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EIGHT MAMMARY GLAND TUMORS ARISING
IN INBRED MICE

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ARTHUR MOSHER CLOUDMAN

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine



INTRODUCTION

The question of the nature of cancer is now being attacked in many fields of research. Tumor tissue transplantation is one of these avenues of approach. A considerable mass of literature has been published on different phases of tumor transplantation, but the findings of only a few investigators bear directly upon the problem reported in this communication. These points of interest were demonstrated largely through the use of genetically controlled stocks of experimental animals. There is little need of taking the time and space to report the findings here. Those who wish to read historical reviews of the literature will find the reports of these investigators thoroughly reviewed by Sailer (1900), Woglom (1913), and recently by Bittner (1931).

STATEMENT OF PROBLEM

The study reported here is one of mammary gland tumors which arose spontaneously in old breeding female mice. These animals were from a derivative of a strain of albino *Mus musculus* which Dr. H. J. Bagg started to inbreed in 1912. He bred the members of this stock for several generations by making pen matings, *i.e.*, by the retention of one male and several females out of the young from the same pen. A certain degree of inbreeding must have been produced by this method. Strong secured representatives of this race in 1920 and since then has bred them as a separate strain maintained by strict brother-to-sister matings. This race was called the A stock. From the time that Strong received them up to the present time,

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many of the old breeding females were observed to develop spontaneous tumors, usually carcinoma of the mammary gland. Several attempts were made to transplant these tumors in 1920, and a large number of A stock mice were used, but no individual of this stock ever grew any of these early transplants.

After eight years Strong secured the thirtieth generation of inbreeding in his laboratory. This, according to Pearl (1914) and Jennings (1916), meant that the stock was as nearly genetically homogenous as mammals can be made to be. During this interval no spontaneous tumors originating in A stock females were used for transplantation.

However, in September 1928, female 14905 had a carcinoma removed, and she and her brother, 14906, were given transplants of this tumor. Both grew the transplants progressively. A series of tumors from A stock females were then transplanted into other A stock mice. All individuals from this original stock proved to be 100 per cent susceptible to all the transplanted mammary gland tumors from females of the A stock.

Of these tumors, eight different ones were selected for study and have been continued to date. This experiment is an attempt to clear up the following points:

1. Are the spontaneous tumors arising in the breasts of females of this inbred A line histologically alike?
2. Is the transplantation inheritance the same in all cases?
 - (a) Does it involve the same number of susceptibility factors for each tumor used?
 - (b) Are the factors involved in the susceptibility to the different tumors identical in any or all cases?
 - (c) Are the same susceptibility factors always necessary for the continued growth of any particular tumor?
3. Is there a definite relationship between the histology of these transplanted tumors and the number of mendelian units underlying susceptibility to them?
4. Does the nearness of the pedigree relationship of the individuals giving rise to these transplanted tumors tend to simplify the analysis of the number and identity of the susceptibility factors involved in the growth of each tumor?

MATERIALS

Stocks Used

- (a) *The A stock*, whose members showed high susceptibility to the tumors used. This stock has already been described.

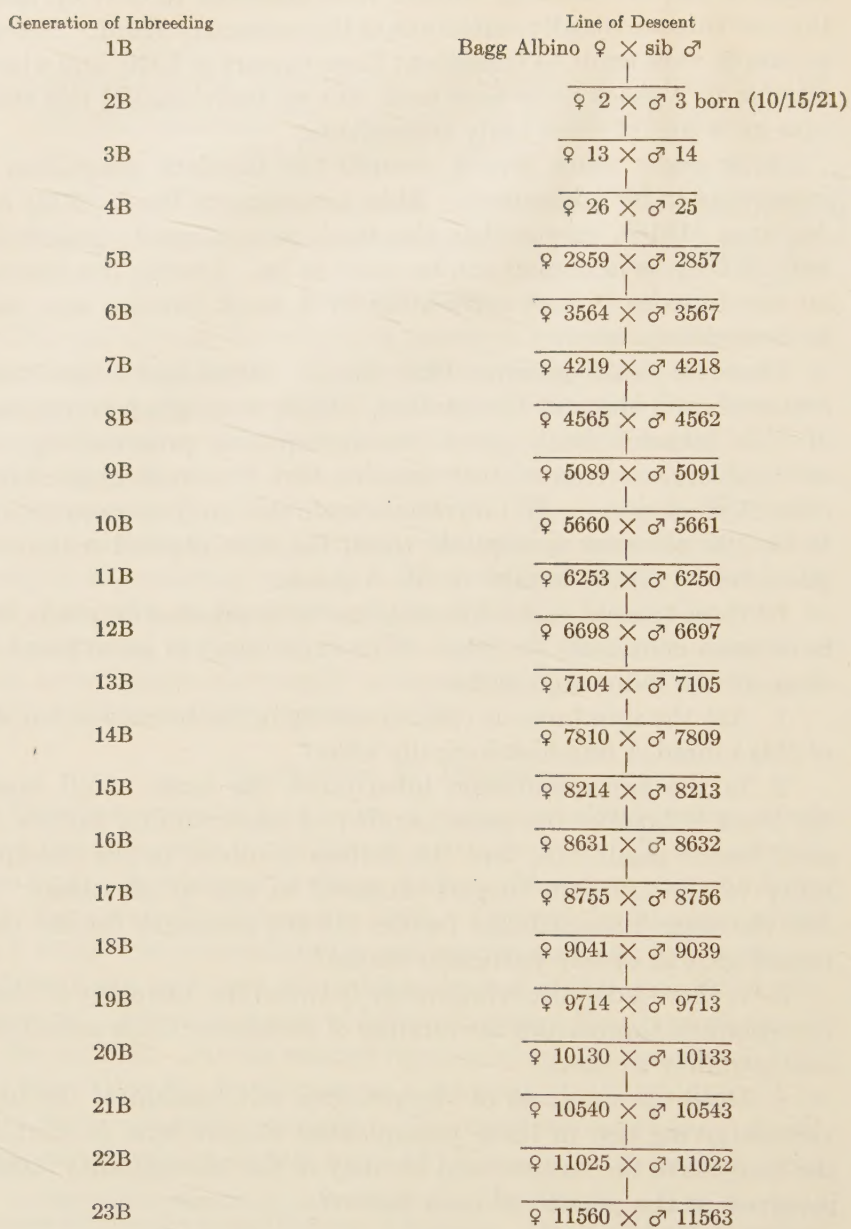


FIG. 1. PEDIGREE OF A STOCK INDIVIDUALS SHOWING THE FIRST TWENTY-THREE GENERATIONS OF BROTHER-TO-SISTER MATINGS

- (b) *The D stock*, whose members showed low susceptibility to the tumors used. This race is even more inbred than the A stock, and is directly descended by brother-to-sister matings from a line of *Mus musculus* started by Little at Harvard University in 1909. This is a dilute brown or silver fawn stock of mice designated here by the letters dBr or D.

In 1919 the dilute brown colony was transferred to Strong, who kept a part of this stock and returned the rest to Little a year later. Since that time the two derivatives have been kept as separate lines: one in Little's laboratory by W. S. Murray, and one in Strong's laboratory. The latter dilute brown mice are in this

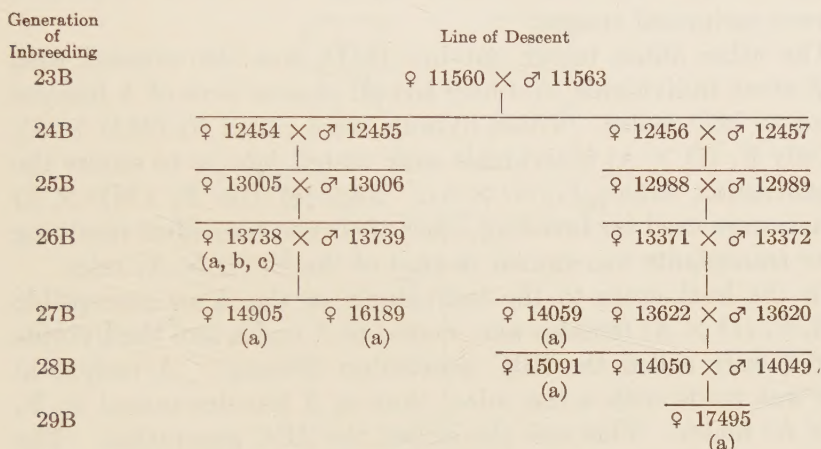


FIG. 2. RELATIONSHIP OF THE FEMALES OF A STOCK PRODUCING THE SPONTANEOUS TUMORS USED FOR TRANSPLANTATION IN THIS EXPERIMENT

Small letters in parentheses denote the spontaneous tumors used for inoculation.

paper called the D stock, or Strong's dilute brown colony, and the former, the MD stock or Murray's dilute brown colony. Here, as in the A stock, only brother-to-sister matings have been used since the beginning, and careful records have been kept.

The members of both A and D stocks are sufficiently inbred to be suitable for this experiment, for theoretically all the individuals of either race should be homozygous for almost all the factors not lethals carried by that particular race.

The A stock individuals were used in this experiment (1) to furnish the tumor tissue, (2) to continue the tissues by transplantation, and (3) for making hybrid generations to receive transplantations. All are descended from female 11560 of the twenty-third generation of brother-to-sister matings (Figs. 1 and 2).

(c) *The N stock*, another low susceptibility race. A dilute brown piebald strain of *Mus musculus* not related to the D stock or the MD stock was also secured from Strong. This stock had been inbred by brother-to-sister matings in his laboratory for ten generations.

Crosses Made

(a) The A stock individuals were crossed with members of the D stock and the F_1 ($D \times A$) generation secured. Hybrids were made by only one type of cross; that is, by using A females and D males. A reciprocal cross was not made because of the small number of D females obtainable and because of the fact that all previous work with transplanted tumors showed no difference between reciprocal crosses.

The other dilute brown sub-line, MD, was also crossed with the A stock individuals, and here too all crosses were of A females mated to MD males. These hybrids were called F_1 ($MD \times A$).

Only F_1 ($D \times A$) individuals were mated *inter se* to secure the F_2 generation called F_2 ($D \times A$). None of the F_1 ($MD \times A$) animals were used for breeding, since their response after receiving tumor transplants was similar to that of the F_1 ($D \times A$) mice.

In the back-cross to the individuals of the A or susceptible stock, F_1 ($D \times A$) females were mated to A males and the hybrids secured were called the ZBC generation (Strong). A reciprocal cross was made with a few mice; that is, A females mated to F_1 ($D \times A$) males. This was also called the ZBC generation. The back-cross to the low susceptibility or D stock was made by using F_1 ($D \times A$) females mated to D males. This was called the 1BC generation (Strong). The F_1 ($D \times A$) males were needed for transplantation, so very few were used for breeding except to secure the F_2 ($D \times A$) generation.

Fig. 3 shows the types of crosses employed to secure the hybrid generations. The cross of the D stock individuals with those of the A stock is the only type shown here. By substituting the letters MD or N for the letter D this figure does equally well for the $MD \times A$ and $N \times A$ crosses.

Neoplasms Used

(a) The neoplasms used are designated by the serial number of the female which gave rise to them. Her serial number is used along with a small letter to designate whether one or more spontaneous tumors from the same individual are employed, as well

as the order in which the multiple neoplasms used occurred spontaneously. For example, female 13738 had three spontaneous tumors which arose independently of each other. All three are used in this experiment. The first spontaneous tumor to be observed was called 13738a, the second became 13738b, and the third 13738c (Fig. 2).

Six A females furnished the eight different spontaneous tumors used. These were female 13738, female 14059, female 15091, female 14905, female 16189, and female 17495, all descended from female 11560 of the twenty-third generation of pedigreed inbreeding. As shown by Fig. 2, they are in two lines of descent (but after such intense inbreeding they should differ very little from

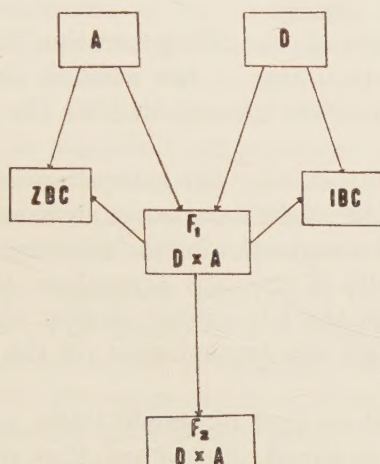


FIG. 3. CROSSES MADE BETWEEN INDIVIDUALS OF STOCKS A AND D AND THEIR HYBRIDS

each other genetically). Only the parents and the females furnishing tumors are shown; none of the other litter mates are included. The list of tumors used follows.

(1) Female 13738 gave rise to three spontaneous tumors which appeared independently of each other. On Dec. 4, 1928, 13738a was observed just anterior to the right iliac region. On the same date a smaller mass, 13738b, was recorded as lying just posterior to the left axillary region. The female was in the twenty-sixth generation of brother-to-sister matings (or in the 26B generation) and she was fourteen and one-half months of age. When she was killed, on Dec. 11, 1928, a third mass, 13738c, was found posterior to the left iliac region. These tumors were all transplanted for the first time on Dec. 11, 1928.

(2) Female 14905 was a daughter of the above female, 13738, and was in the 27B generation. At eight and one-half months of age she developed a tumor which was closely attached to the left thigh. This tumor, 14905a, was first seen Oct. 3, 1928, and was transplanted for the first time into female 14905 (autotransplant) and into a brother, 14906, on Oct. 8, 1928. It was the first A stock tumor to be successfully transplanted.

(3) Female 16189 was another daughter of female 13738 and was also in the 27B generation. At eight months of age she developed a tumor to the left of her external genitalia. This mass, 16189a, was transplanted for the first time on Dec. 11, 1928.

The other three females fall into another, but closely related, line of descent (See Fig. 2).

(4) Female 14059 of the 27B generation developed a tumor, 14059a, on the ventral side of her neck at eleven and one-half months of age. This was transplanted for the first time on Nov. 19, 1928.

(5) Female 15091 of the 28B generation developed a tumor, 15091a, anterior to the right shoulder at eight and one-half months of age. This was transplanted for the first time on Dec. 11, 1928.

(6) Female 17495 of the 29B generation developed a tumor, 17495a, posterior to the left axillary region when she was seven months of age. This was transplanted for the first time on Dec. 11, 1928.

Female 14059 is an aunt of female 15091 and a great-aunt of female 17495 but, as already mentioned, they should differ little if any genetically from females 13738, 14905 and 16189 (see Fig. 2).

(b) *Pathological Diagnoses of the Tumors Used.*² A carcinoma of the breast is a malignant overgrowth of the parenchymatous or epithelial cells of the mammary gland. One of the classifications of carcinoma is based upon the quantitative relationships existing between the stroma and the parenchymatous cells. A carcinoma consisting almost entirely of parenchyma cells is called a medullary carcinoma. This is one of the most malignant forms of carcinoma of the breast. Mallory (1914) states that "cancers of the breast grow most often with cells packed in solid masses, which in sections appear to be arranged in alveoli, but are really all more or less intimately connected together. The cell masses may be large or small and surrounded by little or much stroma."

² I am indebted to the late Dr. A. S. Warthin of the University of Michigan Medical School for these diagnoses.

The common practice in pathological diagnosis is to consider two or three sections of the tumor, taken at random, as characteristic of the whole tumor. However, according to Ewing (1928), several structural types of true cancer are frequently combined in the same tumor. This will often explain the differences in quantitative relationships between the stroma and the parenchyma in the different transplant generations of the various tumors.

A sample of each of the eight spontaneous tumors was sectioned for pathological diagnosis and at various transplant generations additional samples were taken for comparison with the original growth.

Tumors from female 13738: (1) Original growth 13738a was a medullary adenocarcinoma of the mammary gland, in which a few parenchyma cell areas retain the original gland-like structure. (2) Original growth 13738b was a medullary carcinoma of the mammary gland. (3) Original growth 13738c showed most of its parenchyma cells in gland-like arrangement and was called an adenocarcinoma with hemorrhagic necrosis. All three tumors from this female were thus carcinomata of the mammary gland.

Subsequent diagnoses of 13738a show the following:

(1) In the 15th transplant generation tissue from 13738a, taken Nov. 18, 1929, was diagnosed as a medullary carcinoma.

(2) In the 25th transplant generation, tissue of this tumor, taken April 26, 1930, was diagnosed as a medullary carcinoma.

Subsequent diagnoses of 13738b showed:

(1) In the 8th transplant generation, the sample, taken June 20, 1929, was a medullary carcinoma like the original.

(2) In the 10th transplant generation, the sample, taken Aug. 18, 1929, had the same diagnosis.

(3) In the 12th transplant generation, samples were taken from the same tumor growing in two different hosts, Sept. 21, 1929; and while one tissue sample was a medullary carcinoma like the original, the other sample was much more gland-like and was diagnosed as an adenocarcinoma.

(4) In the 16th transplant generation the tissue, taken Dec. 10, 1929, was diagnosed as a medullary carcinoma.

(5) In the 27th transplant generation, the tissue, taken April 13, 1930, was diagnosed, like the original, as a medullary carcinoma.

Subsequent diagnoses of 13738c were:

(1) In the 6th transplant generation, the tissue, taken April 13, 1929, was diagnosed as a medullary carcinoma.

(2) In the 16th transplant generation, the tissue, taken Nov. 12, 1929, was also diagnosed as a medullary carcinoma.

(3) In the 25th transplant generation, the tissue, taken April 24, 1930, was diagnosed as a medullary adenocarcinoma.

Tumor 14905a from female 14905: The original growth was a medullary adenocarcinoma with hemorrhagic necrosis.

Subsequent diagnoses were:

(1) In the 8th transplant generation, tissue, taken April 8, 1929, was diagnosed as a medullary carcinoma.

(2) In the 13th transplant generation, tissue, taken Aug. 19, 1929, was called a medullary carcinoma.

(3) In the 21st transplant generation, tissue, taken Dec. 13, 1929, showed medullary adenocarcinomatous structure.

(4) In the 27th transplant generation, tissue, taken May 3, 1930, showed medullary adenocarcinomatous structure.

Tumor 14059a from female 14059: The original growth was a medullary carcinoma with hemorrhagic necrosis.

Subsequent diagnoses were:

(1) In the 6th transplant generation, tissue, taken April 8, 1929, was diagnosed as the same as the original.

(2) In the 12th transplant generation, tissue, taken Aug. 17, 1929, was diagnosed as the same as the original.

(3) In the 16th transplant generation, tissue, taken Nov. 20, 1929, was diagnosed as the same as the original.

(4) In the 23rd transplant generation, tissue, taken April 27, 1930, was diagnosed as medullary carcinoma without hemorrhagic necrosis.

Tumor 15091a from female 15091: The original tumor was diagnosed as a medullary adenocarcinoma of the mammary gland.

Subsequent diagnoses were:

(1) In the 12th transplant generation, tissue, taken Oct. 7, 1929, was diagnosed as the same as the original tumor.

(2) In the 13th transplant generation, tissue, taken Oct. 25, 1929, was diagnosed as a medullary carcinoma.

(3) In the 20th transplant generation, tissue, taken May 3, 1930, was diagnosed as a medullary carcinoma.

Tumor 16189a from female 16189: The original tumor was diagnosed as a medullary adenocarcinoma.

Subsequent diagnoses were:

(1) In the 13th generation of tissue transfer, the section, taken Oct. 25, 1929, was diagnosed as a medullary carcinoma.

(2) In the 21st tissue generation, tissue, taken March 15, 1930, was a medullary carcinoma.

Tumor 17495a from female 17495: The original tumor was diagnosed as an adenocarcinoma of the mammary gland.

Subsequent diagnoses were:

(1) In the 9th transplant generation, tissue, taken Aug. 19, 1929, was diagnosed as a medullary adenocarcinoma.

(2) In the 13th transplant generation, tissue, taken Nov. 12, 1929, was diagnosed as medullary carcinoma.

(3) In the 22nd transplant generation, tissue, taken April 27, 1930, was a medullary carcinoma.

To summarize: all eight of these tumors are carcinomata of the mammary gland and they would probably be histologically indistinguishable should they be compared throughout.

METHODS

The trocar method of transplantation was employed, since Tyzzer, Little, Strong, and Bittner have previously used this with very satisfactory results in their genetic studies of tumor tissue transplantation. In this present work a separate trocar was used for each tumor and all instruments were boiled for forty-five minutes before they were used.

In the segregating hybrid generations two, three, and sometimes four different tumors were transplanted at the same time into the same hosts. The right and left axillary regions received the tissue transplants subcutaneously and when more than two implants were made into a host the inguinal regions were also used.³ At the end of a week the sites of implantation were palpated and all records of growths and failures of growth were kept on coordinate paper. Weekly observations were continued over a period of six weeks. At the end of three weeks all negative individuals were reinoculated. All mice which showed temporary growths that later completely regressed were grouped with the negative individuals.

The mice to be inoculated were weaned and the sexes segregated at four weeks of age. Inoculations were made at six to ten weeks and in this way any complications arising from pregnancy or age differences were avoided. Food and housing conditions were the same throughout.

³ Bittner (1930) has shown that the axillary and iliac regions are equally susceptible to multiple transplants from the same tumor tissue.

RESULTS

I. Susceptibility of the Individuals of the Stocks Used and of Their Hybrids

Before giving the results in full, the multiple factor hypothesis of Little (Little, 1914; Little and Tyzzer, 1916; Little 1920) will be given, with its expected application to the observed data. It has been demonstrated that susceptibility is a physiological dominant. The multiple factor hypothesis means that susceptibility is due to the simultaneous presence of multiple mendelian factors coming from the susceptible parent stock. A single dose, or heterozygous condition, of all the factors needed for susceptibility is enough for continued growth of the tumor tissue. This condition would be found in the F_1 generation. In the gametogenesis of animals possessing the factor complex in a heterozygous condition (as in the F_1 individuals) the factors for susceptibility are distributed according to random mendelian segregation, no case of linkage having been completely demonstrated. A gamete formed with any one of the necessary factors in a recessive state will not produce a susceptible animal unless it meets, in the process of fertilization, a gamete which carries the dominant allelomorph of this factor.

For this reason the F_2 ($D \times A$) and the 1BC generations are made up of susceptible and non-susceptible individuals in definite ratios which vary with the types of mice and with the tumors used. These are, therefore, the most important generations, and as large numbers of individuals as could be secured in these two generations were used in this tumor transplantation work.

On the other hand, the ZBC generation is formed from fusion of gametes of the susceptible and essentially homozygous A stock individuals with gametes from the F_1 ($D \times A$) individuals. All of the ZBC generation should be susceptible, for all the A stock gametes carry the necessary factor complex in a dominant condition.

To summarize the expectation concerning the susceptibility of the hybrid generations, we may say that all F_1 ($D \times A$) and ZBC individuals should be as susceptible as the susceptible A stock individuals; while the F_2 ($D \times A$) and 1BC animals should segregate into ratios of susceptibles to non-susceptibles which are definite for each tumor.

Each of the eight tumors used in this experiment was transplanted into eight different types of animals. Of these types,

three were inbred lines and the other five were hybrid generations. The inbred lines were the A stock, the D stock, and the MD stock. The A stock individuals were highly susceptible and readily grew the tumors from A stock individuals such as were used in this experiment. On the other hand, the D stock individuals showed very low susceptibility except with two tumors. These exceptional tumors are of peculiar interest and are taken up separately. Individuals of all four hybrid generations of ($D \times A$) gave the expected results with the exception of animals carrying the two tumors which grew in some of the D stock mice. The MD stock individuals gave results quite similar to those of the D stock, and the F_1 ($MD \times A$) animals reacted like the $F_1(D \times A)$ individuals. (For the relationship of these types of individuals see Fig. 3.)

In addition, tumor 13738b was transplanted into individuals of a non-susceptible N stock and its F_1 ($N \times A$) and F_2 ($N \times A$) individuals.

The eight carcinomata of the mammary gland used for transplantation fall into three general groups according to their reactions when transplanted into the above types of animals.

Group I contains those tumors which gave the simple results in all types of animals receiving transplants. This group is further subdivided into:

(a) Tumors that gave a nearly constant ratio of susceptible to non-susceptible animals in the F_2 ($D \times A$) and 1BC generations throughout the entire series of experiments. These tumors were 13738a, 13738c, 14059a, and 14905a.

(b) One tumor which, during the process of transplantation, become less specific, so that the early data show a different ratio of susceptibility in both F_2 ($D \times A$) and 1BC individuals from that collected later. This tumor was 17495a.

Group II contains a single tumor which increased in size more slowly than the other tumors, did not grow as well as expected even in the susceptible A stock mice, and showed by the F_2 ($D \times A$) and 1BC generations that too large a number of genetic factors were involved in determining susceptibility to it to be of much value in a comparative study. This tumor was 16189a.

Group III contains two tumors which showed clear cut reactions in the A stock individuals and the F_1 ($D \times A$) and ZBC generations but gave distorted ratios in individuals of the D stock and its segregating hybrids. These tumors were 13738b and 15091a.

Types of Mice	Observed Reactions		Expected Reaction for Number of Mice			Difference between Observed and Expected Ratios	Total Mice
	Ratios	Per Cent Negative	Factors Needed	Expected Ratios	Per Cent Negative		
A	210.00+; 1.00—	00.47 ± 0.31		211.00+; 0.00—	00.00	00.47%	211
D	0.00+; 96.00—	100.00		0.00+; 96.00—	100.00	00.00%	96
MD	1.00+; 109.00—	99.09 ± 0.60		0.00+; 110.00—	100.00	00.00%	110
F ₁ (D × A)	106.00+; 0.00—	00.00		106.00+; 0.00—	00.00	00.91%	106
F ₁ (MD × A)	94.00+; 0.00—	00.00		94.00+; 0.00—	00.00	00.00%	94
ZBC	103.00+; 0.00—	00.00		103.00+; 0.00—	00.00	00.00%	103
F ₂ (D × A)	150.00+; 144.00— ± 5.78	48.97 ± 1.97	1	220.50+; 73.50— ± 5.00	25.00 ± 1.70	23.97% ± 2.60 or 9.21 × P.E.	294
			2	165.37+; 128.63— ± 5.73	43.75 ± 1.95	5.22% ± 2.77 or 1.88 × P.E.	
			3	124.03+; 169.97— ± 5.71	57.81 ± 1.94	8.84% ± 2.76 or 3.20 × P.E.	
1BC	52.00+; 153.00— ± 4.20	74.63 ± 2.05	1	102.50+; 102.50— ± 4.82	50.00 ± 2.35	24.63% ± 3.11 or 7.91 × P.E.	205
			2	51.25+; 153.75— ± 4.17	75.00 ± 2.03	0.37% ± 2.88 or 0.012 × P.E.	
			3	25.62+; 179.38— ± 3.19	87.50 ± 1.55	12.87% ± 2.57 or 5.02 × P.E.	

FIG. 4. REACTIONS BETWEEN TUMOR 13738a AND TYPES OF MICE EMPLOYED

GROUP I: Tumors which gave simple results in all types of animals which received transplants.

(a) Tumors which gave nearly constant ratios in individuals of the F_2 ($D \times A$) and 1BC generations.

1. The reactions between tumor 13738a and the types of mice receiving the tumor are shown in Fig. 4. In the first six types employed there were but two exceptional individuals, one in the A stock and one in the MD stock. The exceptional A mouse was male C1 1769, who was the product of thirty-three generations of unbroken brother-to-sister matings. He was also negative to tumors 13738b and 14059a. This mouse had two susceptible brothers. Large enough numbers of A and MD stock individuals

Number of Factors	Factorial Composition of F_1 ($D \times A$)	Expectations in F_2 ($D \times A$)	Ratio in F_2 ($D \times A$)	Per Cent Negatives F_2 ($D \times A$)	Expectations in 1BC	Per Cent Negatives in 1BC
1	Aa	3+ : 1-	1+ : 0.33-	25.00	1+ : 1-	50.00
2	AaBb	9+ : 7-	1+ : 0.78-	43.75	1+ : 3-	75.00
3	AaBbCc	27+ : 37-	1+ : 1.37-	57.81	1+ : 7-	87.50
4	AaBb...Dd	81+ : 175-	1+ : 2.16-	68.35	1+ : 15-	93.75
5	AaBb...Ee	243+ : 781-	1+ : 3.21-	76.27	1+ : 31-	96.87
6	AaBb...Ff	729+ : 3367-	1+ : 4.61-	82.20	1+ : 63-	98.43
7	AaBb...Gg	2187+ : 14197-	1+ : 6.49-	86.65	1+ : 127-	99.21
8	AaBb...Hh	6561+ : 58975-	1+ : 9.004-	89.99	1+ : 255-	99.60
9	AaBb...Ii	19,683+ : 242,461-	1+ : 12.32-	92.49	1+ : 509-	99.80
10	AaBb...Jj	59,049+ : 989,527-	1+ : 16.75-	94.36	1+ : 1019-	99.90
11	AaBb...Kk	177,147+ : 4,017,157-	1+ : 22.67-	95.77	1+ : 2039-	99.95
12	AaBb...Ll	531,441+ : 16,245,775-	1+ : 30.56-	96.83	1+ : 4079-	99.97
13	AaBb...Mm	1,594,323+ : 65,514,541-	1+ : 41.09-	97.62		

FIG. 5. EXPECTED PROPORTIONS OF SUSCEPTIBLE (+) AND NON-SUSCEPTIBLE (-) INDIVIDUALS IN THE F_2 ($D \times A$) AND 1BC GENERATIONS

were used so that the differences between the observed and expected ratios are not significant. We see that individuals of the F_1 ($D \times A$), F_1 (MD $\times$ A), and ZBC types were 100 per cent susceptible to tumor 13738a and carried the necessary susceptibility factors in a homozygous dominant (ZBC) or a heterozygous condition (F_1 ($D \times A$), F_1 (MD $\times$ A) and ZBC). The A individuals were practically 100 per cent susceptible (99.53 per cent). On the other hand, the individuals of the D stock were 100 per cent negative and those of the MD stock practically 100 per cent negative to tumor 13738a. The numbers in each group are significantly large.

The F_2 ($D \times A$) and 1BC generations are the important types

Types of Mice	Observed Reactions		Expected Reaction for Number of Mice			Difference between Observed and Expected Ratios	Total Mice
	Ratios	Per Cent Negative	Factors Needed	Expected Ratios	Per Cent Negative		
A	155.00+ : 0.00 -	00.00		155.00+ : 0.00 -	00.00	00.00%	155
D	0.00+ : 75.00 -	100.00		0.00+ : 75.00 -	100.00	00.00%	75
MD	1.00+ : 123.00 -	99.19 $\pm$ 0.53		0.00+ : 124.00 -	100.00	00.81%	124
F ₁ (D $\times$ A)	121.00+ : 0.00 -	00.00		121.00+ : 0.00 -	00.00	00.00%	121
F ₁ (MD $\times$ A)	39.00+ : 0.00 -	00.00		39.00+ : 0.00 -	00.00	00.00%	39
ZBC	87.00+ : 1.00 -	1.13 $\pm$ 0.75		88.00+ : 0.00 -	00.00	1.13%	88
F ₂ (D $\times$ A)	190.00+ : 171.00 -	47.36 $\pm$ 1.77	1	270.75+ : 90.25 -	25.00 $\pm$ 1.53	22.36% $\pm$ 2.33 or 9.59 $\times$ P.E.	361
			2	203.06+ : 157.94 -	43.75 $\pm$ 1.75	3.61% $\pm$ 2.48 or 1.45 $\times$ P.E.	
			3	152.30+ : 208.70 -	57.81 $\pm$ 1.75	10.45% $\pm$ 2.48 or 4.21 $\times$ P.E.	
1BC	36.00+ : 150.00 -	80.64 $\pm$ 1.94	1	93.00+ : 93.00 -	50.00 $\pm$ 2.46	30.64% $\pm$ 3.13 or 9.78 $\times$ P.E.	186
			2	46.50+ : 139.50 -	75.00 $\pm$ 2.13	5.64% $\pm$ 2.88 or 1.95 $\times$ P.E.	
			3	23.25+ : 162.75 -	87.50 $\pm$ 1.63	6.86% $\pm$ 2.53 or 2.71 $\times$ P.E.	
			4	11.63+ : 174.37 -	93.75 $\pm$ 1.19	13.11% $\pm$ 2.27 or 5.77 $\times$ P.E.	

Fig. 6. REACTIONS BETWEEN TUMOR 13738c AND TYPES OF MICE EMPLOYED

of animals, for they show the probable number of susceptibility factors involved in the growth of the transplanted tumor in question. These two types are of further value in that they serve as a reliable check upon each other. Fig. 5 shows the expected proportions of susceptible individuals in the F_2 ($D \times A$) and 1BC generations when 1 to 13 susceptibility factors are involved.

We will first give the results of inoculating the tumor 13738a into individuals of the F_2 ($D \times A$) generation. A total of 294 animals were used in this group. Of these, 150 carried, either in a heterozygous or homozygous dominant condition, all the multiple factors necessary for the continued growth of tumor 13738a. The remaining 144 animals probably carried one or more of the allelomorphs of these same factors in the homozygous recessive condition and for this reason failed to grow the transplanted tumor.

The observed ratio is compared with the expectations for this number of individuals when one, two, and three susceptibility factors are needed for the growth of tumor 13738a. This is shown in Figs. 4 and 5. It can be readily seen that the expected ratios for one and for three susceptibility factors are significantly different from the observed ratio; while the expected ratio for two susceptibility factors differs from the observed ratio by only 1.88 times the probable error. This is not a significant difference and the observed F_2 ($D \times A$) data give a strong indication that two susceptibility factors are needed for the growth of tumor 13738a.

When we examine the data for the 205 1BC individuals which received transplants of tumor 13738a, we see a verification of the above assumption. The probability that one or three susceptibility factors are needed is definitely eliminated. Furthermore, the observed ratio is so close to the expected ratio when two susceptibility factors are needed that there is a difference of but 0.012 times the probable error between them.

The above data show that the need of the presence of two susceptibility factors would very readily explain the ratios obtained with individuals of both the F_2 ($D \times A$) and 1BC generations that have received transplants of tumor 13738a. These factors were contributed by the individuals of the A stock.

2. The reactions between the second tumor of this group, 13738c, and the types of animals receiving it are shown in Fig. 6. Here the first six types of mice employed show two exceptional individuals. One of these was an animal of the pure MD stock and the other an individual of the ZBC generation. This excep-

Types of Mice	Observed Ratio		Expected Reaction for Number of Mice			Difference between Observed and Expected Ratios	Total Mice
	Ratios	Per Cent Negative	Factors Needed	Expected Ratios	Per Cent Negative		
A	159.00+ : 1.00- ± 0.66	00.62 ± 0.62		160.00+ : 0.00-	00.00	00.62%	160
D	0.00+ : 66.00-	100.00		0.00+ : 66.00-	100.00	00.00%	66
MD	0.00+ : 117.00-	100.00		0.00+ : 117.00-	100.00	00.00%	117
F ₁ (D × A)	92.00+ : 0.00-	00.00		92.00+ : 0.00-	00.00	00.00%	92
F ₁ (MD × A)	56.00+ : 0.00-	00.00		56.00+ : 0.00-	00.00	00.00%	56
ZBC	69.00+ : 0.00-	00.00		69.00+ : 0.00-	00.00	00.00%	69
F ₂ (D × A)	60.00+ : 159.00- ± 4.45	72.60 ± 2.03	3	92.39+ : 126.61- ± 4.92	57.81 ± 2.24	14.79% ± 3.02 or 4.89 × P.E.	219
			4	69.30+ : 149.70- ± 4.64	68.35 ± 2.12	4.25% ± 2.93 or 1.45 × P.E.	
			5	51.97+ : 167.03- ± 4.24	76.27 ± 1.93	3.67% ± 2.80 or 1.31 × P.E.	
			6	38.98+ : 180.02- ± 3.81	82.20 ± 1.74	9.60% ± 2.67 or 3.59 × P.E.	
			2	29.00+ : 87.00- ± 3.14	75.00 ± 2.70	16.37% ± 3.21 or 5.09 × P.E.	116
		91.37 ± 1.75	3	14.50+ : 101.50- ± 2.40	87.50 ± 2.06	3.87% ± 2.70 or 1.43 × P.E.	
			4	7.25+ : 108.75- ± 1.75	93.75 ± 1.50	2.38% ± 2.30 or 1.03 × P.E.	
			5	3.63+ : 112.37- ± 1.26	96.87 ± 1.08	5.50% ± 2.05 or 2.68 × P.E.	
IBC	10.00+ : 106.00- ± 2.03		6	1.82+ : 114.18- ± 0.89	98.43 ± 0.76	7.06% ± 1.90 or 3.71 × P.E.	

Fig. 7. REACTIONS BETWEEN TUMOR 14059a AND TYPES OF MICE EMPLOYED

tional animal makes the mice of the MD stock 99.19 per cent negative, which is probably not significantly different from the expected 100 per cent negative. In the case of the one exceptional ZBC animal death occurred soon after the third observation, before the results of reinoculation could be determined. The individuals of the A, F_1 ($D \times A$), and F_1 ($MD \times A$) types were 100 per cent susceptible, and the D type individuals were 100 per cent non-susceptible, as expected.

When we consider the data for the 361 F_2 ($D \times A$) individuals which received transplants of tumor 13738c, we find that probably more than one and less than three susceptibility factors are involved in the successful growth of this tumor. The observed ratio differs by only 1.45 times the probable error from the expected ratio when two susceptibility factors are needed. The need of the presence of two susceptibility factors for the progressive growth of tumor 13738c would explain the results obtained with the individuals of the F_2 ($D \times A$) generation.

Referring to the 1BC data, we can readily see that more than one but less than four susceptibility factors are probably needed for the growth of tumor 13738c. The observed ratio differs by but 1.95 times the probable error from the expected ratio when two susceptibility factors are involved. This is probably not a significant difference. However, the observed ratio differs by but 2.71 times the probable error from the expected ratio when three susceptibility factors are needed. This is approaching significance, but the possibility that three susceptibility factors are needed is not excluded.

The data for the individuals of the 1BC generation who received transplants of tumor 13738c show that the need of the presence of two or three (probably two) susceptibility factors for continued tumor growth would explain the data obtained. This substantiates the finding with the individuals of the F_2 ($D \times A$) generation.

3. The next tumor in this group is 14059a. In the first six types of animals employed there is but one exceptional individual (Fig. 7). This was male Cl 1769 of the A stock, who has already been discussed under tumor 13738a. Even if this one individual was truly negative, there would be a deviation of only 00.62 per cent from the expected 100 per cent susceptible. This difference is probably not significant.

By scanning the data for the F_2 ($D \times A$) and 1BC generations we see that more susceptibility factors are involved here than were

Types of Mice	Observed Ratios		Expected Reaction for Number of Mice			Difference between Observed and Expected Ratios	Total Mice
	Ratios	Per Cent Negative	Factors Needed	Expected Ratios	Per Cent Negative		
A	206.00+ : 0.00-	00.00		206.00+ : 0.00-	00.00	00.00%	206
D	0.00+ : 67.00-	100.00		0.00+ : 67.00-	100.00	00.00%	67
MD	0.00+ : 107.00-	100.00		0.00+ : 107.00-	100.00	00.00%	107
F ₁ (D × A)	105.00+ : 0.00-	00.00		105.00+ : 0.00-	00.00	00.00%	105
F ₁ (MD × A)	40.00+ : 0.00-	00.00		40.00+ : 0.00-	00.00	00.00%	40
ZBC	91.00+ : 0.00-	00.00		91.00+ : 0.00-	00.00	00.00%	91
F ₂ (D × A)	67.00+ : 154.00- ± 4.60	69.68 ± 2.08	3	93.24+ : 127.76- ± 4.95	57.81 ± 2.25	11.87% ± 3.04	221
			4	69.95+ : 151.05- ± 4.66	68.35 ± 2.10	or 3.90 × P.E. 1.33% ± 2.95	
			5	52.45+ : 168.55- ± 4.26	76.27 ± 1.92	or 0.45 × P.E. 6.59% ± 2.83	
			6	39.34+ : 181.66- ± 3.83	82.20 ± 1.73	or 2.32 × P.E. 12.52% ± 2.70	
			2	21.75+ : 65.25- ± 2.71	75.00 ± 3.11	or 4.63 × P.E. 18.10% ± 3.60	87
	6.00+ : 81.00- ± 1.59	93.10 ± 1.83	3	10.88+ : 76.12- ± 2.07	87.50 ± 2.37	or 5.02 × P.E. 5.60% ± 2.99	
			4	5.44+ : 81.56- ± 1.51	93.75 ± 1.73	or 1.87 × P.E. 0.65% ± 2.51	
			5	2.73+ : 84.27- ± 1.09	96.87 ± 1.24	or 0.25 × P.E. 3.77% ± 2.21	
			6	1.37+ : 85.63- ± 0.78	98.34 ± 0.89	or 1.70 × P.E. 5.33% ± 2.03	
			7	0.69+ : 86.31- ± 0.55	99.21 ± 0.62	or 2.62 × P.E. 6.11% ± 1.93	
						or 3.16 × P.E.	

FIG. 8. REACTIONS BETWEEN TUMOR 14905a AND TYPES OF MICE EMPLOYED

needed by either of the first two tumors. It is also evident that more than three but less than six susceptibility factors are probably needed to explain the observed data for the reactions of the 219 F_2 ($D \times A$) individuals which received implants of tumor 14059a. Furthermore the observed ratio is not significantly different from the expected ratios when either four or five susceptibility factors are needed. An examination of the 1BC data shows that the number of factors needed for susceptibility probably lies between two and six factors. None of the expected ratios for three, four, or five susceptibility factors is significantly different from the observed ratio for the 116 individuals of the 1BC generation which were used with this tumor. The data for the 1BC generation contain fewer individuals than those for the F_2 ($D \times A$) generation, but the findings are quite similar. Probably four factors for susceptibility are needed for the continued growth of tumor 14059a, but it is not impossible that three or five factors are needed.

4. Another tumor in this group is 14905a. By scanning Fig. 8 it will be seen that there were no exceptional animals among the individuals of the first six types of animals which received implants of this tumor.

As to the number of susceptibility factors needed by those individuals which grew this tumor, we find that more than three but less than six of these factors were needed by the 221 F_2 ($D \times A$) mice employed. The ratio expected for this number of animals when four factors are involved differs from the observed ratio by only 0.45 times the probable error. This difference is not significant, and the observed ratio may well be a four-factor ratio. However, the possibility that five factors are needed is not excluded even though the difference here is approaching significance. The 1BC data show that the probable number of susceptibility factors needed may be three, four, five, or even six. The expected ratio for four of these factors is nearest to the observed ratio and differs from it by only 0.25 times the probable error. Considerably fewer individuals of the 1BC generation than of the F_2 ($D \times A$) generation were available for use with this tumor. For this reason the F_2 ($D \times A$) data are probably more valuable than those for the 1BC animals. The mice of the F_2 ($D \times A$) type have shown that the need of the presence of four or five (probably four) susceptibility factors for the continued growth of tumor 14905a will explain the data obtained. The mice of the 1BC type have also shown that four susceptibility factors might easily be the

Types of Mice	Observed Reactions		Expected Reactions for Number of Mice			Difference between Observed and Expected Ratios	Total Mice
	Ratios	Per Cent Negative	Factors Needed	Expected Ratios	Per Cent Negative		
A	211.00+; 0.00—	00.00		211.00+; 0.00—	00.00	00.00%	211
D	1.00+; 93.00— ± 0.66	98.93 ± 0.70		0.00+; 94.00—	100.00	1.07%	94
MD	0.00+; 124.00—	100.00		0.00+; 124.00—	100.00	00.00%	124
F ₁ (D × A)	117.00+; 0.00—	00.00		117.00+; 0.00—	00.00	00.00%	117
F ₁ (MD × A)	62.00+; 0.00—	00.00		62.00+; 0.00—	00.00	00.00%	62
ZBC	67.00+; 0.00—	00.00		67.00+; 0.00—	00.00	00.00%	67
F ₂ (D × A)	249.00+; 123.00— ± 6.11	33.06 ± 1.64	1	279.00+; 93.00— ± 5.63	25.00 ± 1.51	8.06% ± 2.22 or 3.63 × P.E.	372
Total			2	209.25+; 162.75— ± 6.44	43.75 ± 1.73	10.69% ± 2.38 or 4.49 × P.E.	
Original	70.00+; 58.00— ± 3.79 Dec. 11, 1928 to Jan. 1, 1930	45.31 ± 2.96	1	96.00+; 32.00— ± 3.29	25.00 ± 2.57	20.31% ± 3.92 or 5.18 × P.E.	
			2	72.00+; 56.00— ± 3.75	43.75 ± 2.95	1.56% ± 4.17 or 0.37 × P.E.	
			3	54.01+; 73.99— ± 3.76	57.87 ± 2.93	12.50% ± 4.16 or 3.00 × P.E.	
Mutant	179.00+; 65.00— ± 4.65 Feb. 26, 1930 to Oct. 21, 1930	26.63 ± 1.91	1	183.00+; 61.00— ± 4.55	25.00 ± 1.86	1.63% ± 2.66 or 0.61 × P.E.	
			2	137.25+; 106.75— ± 5.22	43.75 ± 2.13	17.12% ± 2.86 or 5.98 × P.E.	
1BC	90.00+; 146.00— ± 5.03	61.86 ± 2.13	1	118.00+; 118.00— ± 5.18	50.00 ± 2.19	11.86% ± 3.05 or 3.88 × P.E.	236
Total			2	59.00+; 177.00— ± 4.48	75.00 ± 1.89	13.14% ± 2.84 or 4.62 × P.E.	
Original	27.00+; 60.00— ± 2.90 Dec. 11, 1928 to Jan. 13, 1930	68.96 ± 3.33	1	43.50+; 43.50— ± 3.14	50.00 ± 3.60	18.96% ± 4.90 or 3.86 × P.E.	
			2	21.75+; 65.25— ± 2.71	75.00 ± 3.11	6.04% ± 4.55 or 1.35 × P.E.	
			3	10.88+; 76.12— ± 2.07	87.50 ± 2.37	18.54% ± 4.08 or 4.54 × P.E.	
Mutant	63.00+; 86.00— ± 4.06 Feb. 13, 1930 to Dec. 1, 1930	57.71 ± 2.73	1	74.50+; 74.50— ± 4.11	50.00 ± 2.75	7.71% ± 3.87 or 1.99 × P.E.	
			2	37.24+; 111.76— ± 3.56	75.00 ± 2.38	17.29% ± 3.62 or 3.77 × P.E.	

FIG. 9. REACTIONS BETWEEN TUMOR 17495a AND TYPES OF MICE EMPLOYED

necessary number required for the growth of this tumor. As stated above, some other possibilities are, however, not excluded as to the number of susceptibility factors needed in the susceptible 1BC mice.

(b) One tumor which during the process of transplantation became less specific so that the early data show a different ratio of susceptibility in both F_2 ($D \times A$) and 1BC generation individuals from those data collected in the last half of the experiment. This tumor, 17495a, showed but one exceptional individual in the first six types of mice employed (Fig. 9). This exceptional individual was a D stock male, B 4889. His pedigree shows strict brother-to-sister matings back to the time that the D stock was brought into Strong's laboratory. The observed ratio even with this one exception does not deviate significantly from the expected 100 per cent non-susceptibility to this tumor.

An inspection of the data for the reactions of the F_2 ($D \times A$) individuals shows that they cannot be explained by assuming that the presence of one or two susceptibility factors is needed by those individuals which grew the tumor. The observed ratio lies between the expected ratios for one and for two susceptibility factors, but it is significantly different from both expected ratios. The number of individuals included is 372, and this should be a large enough number to give a reliable ratio. One explanation of this observed ratio is that it might be composed of two different ratios. A study of the data by experiments covering a period of two years shows a sudden increase in the number of susceptible animals at a point between Jan. 1, 1930, and Feb. 26, 1930. Prior to that time the negative per cents of the observed ratios for the different experiments with F_2 ($D \times A$) individuals fluctuated around 45 to 47 per cent. From Feb. 26, 1930, to Oct. 21, 1930, the negative per cents fluctuated around 26 to 28 per cent. A significant change apparently occurred resulting in the need of fewer susceptibility factors for the successful growth of tumor 17495a. When the data are divided as above, we find that up to Jan. 1, 1930, the observed ratio differs from the expected ratio for two factors by only 0.37 times the probable error. This difference is not significant, and the observed ratio is explainable by assuming that two susceptibility factors were needed by the mice that grew this tumor. Furthermore, the observed ratio is significantly different from the expected ratios for one and for three factors. From Feb. 26, 1930, to Oct. 21, 1930, the observed ratio differs from the expected ratio

Types of Mice	Observed Reactions		Expected Reactions for Number of Mice			Difference between Observed and Expected Ratios	Total Mice
	Ratios	Per Cent Negative	Factors Needed	Expected Ratios	Per Cent Negative		
A	137.00+; 4.00- ± 1.33	2.83 ± 0.95		141.00+; 0.00-	00.00	2.83%	141
D	0.00+; 8.00-	100.00		0.00+; 8.00-	100.00	00.00%	8
MD	0.00+; 128.00-	100.00		0.00+; 128.00-	100.00	00.00%	128
F ₁ (D × A)	38.00+; 0.00-	00.00		38.00+; 0.00-	00.00	00.00%	38
F ₁ (MD × A)	15.00+; 0.00-	00.00		15.00+; 0.00-	00.00	00.00%	15
ZBC	62.00+; 3.00- ± 1.14	4.61 ± 1.75		65.00+; 0.00-	00.00	00.00%	65
F ₂ (D × A)	4.00+; 110.00- ± 1.32	96.49 ± 1.15	8	11.42+; 102.58- ± 2.15	89.99 ± 1.85	4.61% 6.50% ± 2.19 or 2.96 × P.E.	114
			9	8.57+; 105.43- ± 1.89	92.49 ± 1.65	4.00% ± 2.01 or 1.99 × P.E.	
			10	6.43+; 107.57- ± 1.65	94.36 ± 1.44	2.13% ± 1.84 or 1.15 × P.E.	
			11	4.83+; 109.17- ± 1.45	95.77 ± 1.26	0.72% ± 1.70 or 0.42 × P.E.	
			12	3.62+; 110.38- ± 1.26	96.83 ± 1.09	0.34% ± 1.58 or 0.21 × P.E.	
			13	2.71+; 111.29- ± 1.09	97.62 ± 0.95	1.13% ± 1.49 or 0.75 × P.E.	
1BC	0.00+; 101.00-	100.00	8	0.41+; 100.58- ± 0.42	99.60 ± 0.40	0.40%	101
			12	0.03+; 100.97- ± 0.03	99.97 ± 0.03	0.03%	

FIG. 10. REACTIONS BETWEEN TUMOR 16189a AND TYPES OF MICE EMPLOYED

for one susceptibility factor by only 0.61 times the probable error, which is not significant. It differs from the expected ratio for two factors by 5.98 times the probable error. This is a significant difference, for the chances are about 19,000 : 1 (Pearl 1930) that the observed ratio is not dependent upon two susceptibility factors. These data substantiate the hypothesis that the total data are divisible into two definite ratios which clearly indicate a change from a clear cut two-factor ratio to a good one-factor ratio. This change is probably in the nature of a somatic mutation (Strong 1926) in tumor 17495a during the process of transplantation.

This hypothesis is further substantiated by the data obtained with the individuals of the 1BC generation. Here, as with the F_2 ($D \times A$) individuals, the observed ratio appears to be a mixed ratio. It differs significantly from the expected ratios for one and for two susceptibility factors but lies between these two ratios. The data for the 1BC individuals also show a sudden increase in the number of susceptible animals between Jan. 13, 1930, and Feb. 13, 1930. The observed ratio for the early data differs significantly from the expected ratios when one and three susceptibility factors are needed. On the other hand, the expected ratio for two factors differs from the observed early ratio by only 1.35 times the probable error, which is not significant. Two susceptibility factors were probably needed by the 1BC animals previous to Jan. 13, 1930. From Feb. 13, 1930, to Dec. 1, 1930, the observed ratio is significantly different from the ratio expected when two susceptibility factors are needed. There is a difference of 3.77 times the probable error between them. The expected ratio when one factor is involved is quite close to the observed late ratio, with a difference between them of but 1.99 times the probable error.

The data for both the F_2 ($D \times A$) and 1BC individuals strongly indicate that a somatic mutation occurred in tumor 17495a at some time during late January or early February 1930. This is shown in both types of animals by an apparent change from the need of the presence of two susceptibility factors to a condition where only one of these factors is required to make an individual grow the transplanted tumor.

GROUP II: Here is a single tumor which increased in size more slowly than did the seven other tumors, and it did not grow as well as expected in the individuals of the A stock. Evidently a large number of susceptibility factors are involved in the growth of this tumor, 16189a.

The reactions between tumor 16189a and the types of animals receiving it are shown in Fig. 10. Inspection of these data shows that two out of the first six types of individuals employed contain exceptional animals. There were four out of 141 of the A stock individuals which failed to grow transplants of tumor 16189a even after a second implantation of the tissue. These four exceptions show pedigrees of thirty or more generations of inbreeding. One of these mice died four weeks after the start of the experiment and one week after reinoculation. The other three lived for over two and one-half months without developing masses. The individuals of the A stock are nearly 100 per cent susceptible to tumor 16189a.

Three other exceptional animals were in the ZBC generation. These exceptions all occurred in one experiment, where tumors 16189a and 15091a were transplanted into opposite sides of the same animals. The mice were all susceptible to tumor 15091a, and its rapid growth killed all of the animals by the end of the fifth week. The negative mice received second transplants of tumor 16189a at the end of the third week. Their death two weeks later came too soon to allow them to demonstrate their final reaction to tumor 16189a.

The reactions of tumor 16189a when transplanted into the individuals of the F_2 ($D \times A$) and 1BC generations give clear evidence that a large number of susceptibility factors are needed by the individuals which grow this tumor. As seen in Fig. 10, none of the expectations of from eight to fourteen or more susceptibility factors differ significantly from the observed ratio for the reactions of the 114 individuals of the F_2 ($D \times A$) generation. The numbers of individuals used here were not as great as with other tumors, since the growth rate of tumor 16189a was incompatible with that of the other tumors, and multiple transplantations including tumor 16189a were not employed. With this number of individuals the susceptible animals were few, and the loss of one or two would make quite a difference in the data obtained. We see at once that a large number of mice would have to be included before we could tell, even within two factors, how many susceptibility factors must be carried by the animals which grow this tumor.

When we examine the 1BC data, we see further evidence that the above assumption is true. Fig. 5 shows that when more than eight multiple factors are involved the proportion of negative animals rises rapidly in the 1BC generation. When eight factors are needed for susceptibility we would get approximately one suscep-

tible to every 255 non-susceptible animals or a ratio of $1 + : 255 -$. When ten factors are involved, this ratio becomes $1 + : 1019 -$ and when twelve susceptibility factors are needed the ratio increases to $1 + : 4079 -$. Since none of the 1BC individuals grew this tumor, it is out of the question to determine with these data the number of factors needed for its growth. However, Fig. 5 shows us that there is little chance of getting susceptible 1BC mice, since the data for the reaction of the F_2 ($D \times A$) mice indicate that eight to fourteen or more factors are needed. This suggests that even a population of 10,000 mice of the 1BC generation might not be enough to give clear cut results.

These data upon observations of tumor 16189a transplanted into individuals of the F_2 ($D \times A$) and 1BC generations are of interest, for they show that not all the spontaneous tumors from individuals of the inbred A stock are dependent upon only a few susceptibility factors. While carrying along the seven other tumors, it was impossible to work out fully the number of factors involved in susceptibility of tumor 16189a. However, with data from a very large number of transplantations into individuals of the F_2 ($D \times A$) and 1BC generations, this tumor could probably be shown to be like those already taken up, but dependent upon a much larger number of factors.

GROUP III: Here are two tumors which gave the expected reactions in the individuals of the A stock, the F_1 ($D \times A$), F_1 ($MD \times A$), and the ZBC generations, but gave distorted ratios in the animals of the D stock, F_2 ($D \times A$) and 1BC generations.

1. The reactions between tumor 13738b and the types of mice receiving it are shown in Fig. 11. It will be seen here that there are eleven types of individuals employed with this tumor. The first eight types should contain individuals which are either all susceptible or all non-susceptible according to the type under which they are classed. Fig. 11 shows that four of these eight types contain exceptional individuals. In the A stock male C1 1769 failed to grow the tumor. He has been discussed under tumor 13738a. Considering him as a negative mouse gives a difference of only 00.37 per cent between the observed data and the expected 100 per cent susceptible. Very probably this is not significant, for 264 individuals were employed, which is a good sample of the A stock mice. Another exceptional animal was female B 2462 of the ZBC generation, who failed to grow the tumor even upon receiving a second transplant. This gives a difference of but 00.92 per cent

Types of Mice	Observed Reactions		Expected Reaction for Number of Mice			Difference between Observed and Expected Ratios	Total Mice
	Ratios	Per Cent Negative	Factors Needed	Expected Ratios	Per Cent Negative		
A	264.00+ : 1.00- ± 0.66	00.37 ± 0.25		265.00+ : 0.00-	00.00	00.37%	265
D	22.00+ : 48.00- ± 2.61	68.57 ± 3.73	1 recessive	0.00+ : 70.00- 17.50+ : 52.50- ± 2.44	100.00 75.00 ± 3.48	31.43% 6.43% ± 5.10 or 1.26 × P.E.	70
MD			2 recessive	4.37+ : 65.63- ± 1.36	93.75 ± 1.95	21.18% ± 4.20 or 5.99 × P.E.	
N	18.00+ : 131.00- ± 2.69	87.91 ± 1.81		0.00+ : 149.00-	100.00	12.09%	149
F ₁ (D × A)	0.00+ : 22.00-	100.00		0.00+ : 22.00-	100.00	00.00%	22
F ₁ (MD × A)	135.00+ : 0.00-	00.00		135.00+ : 0.00-	00.00	00.00%	135
F ₁ (N × A)	21.00+ : 0.00-	00.00		21.00+ : 0.00-	00.00	00.00%	21
ZBC	79.00+ : 0.00-	00.00		79.00+ : 0.00-	00.00	00.00%	79
F ₂ (D × A)	107.00+ : 1.00- ± 0.66 206.00+ : 102.00- ± 5.56	00.92 ± 0.61 33.11 ± 1.81	1 dominant	108.00+ : 0.00- 231.00+ : 77.00- ± 5.12	00.00 25.00 ± 1.66	00.92% 8.11% ± 2.45 or 3.31 × P.E.	108
Corrected			2 dominant	173.25+ : 134.75- ± 5.86	43.75 ± 1.90	10.64% ± 2.62 or 4.06 × P.E.	308
F ₂ (N × A)	196.45+ : 111.55- ± 5.68 144.00+ : 102.00- ± 5.20	36.21 ± 1.85 41.46 ± 2.11	2 dominant 1 dominant	173.25+ : 134.75- ± 5.86 184.50+ : 61.50- ± 4.58	43.75 ± 1.90 25.00 ± 1.86	7.54% ± 2.65 or 2.84 × P.E. 16.46% ± 2.81 or 5.85 × P.E.	246
IBC			2 dominant 3 dominant	138.38+ : 107.62- ± 5.24 103.79+ : 142.21- ± 5.22	43.75 ± 2.12 57.81 ± 2.12	2.29% ± 2.99 or 0.76 × P.E. 16.35% ± 2.99 or 5.46 × P.E.	
	64.00+ : 135.00- ± 4.44	67.83 ± 2.24	1 dominant	99.50+ : 99.50- ± 4.75	50.00 ± 2.38	17.83% ± 3.26 or 5.45 × P.E.	199
Corrected			2 dominant 3 dominant	49.75+ : 149.25- ± 4.11 24.87+ : 174.13- ± 3.14	75.00 ± 2.06 87.50 ± 1.59	7.17% ± 3.05 or 2.35 × P.E. 19.67% ± 2.74 or 7.17 × P.E.	
	46.55+ : 152.45- ± 4.02	76.60 ± 2.02	2 dominant	49.75+ : 149.25- ± 4.11	75.00 ± 2.06	1.60% ± 2.88 or 0.55 × P.E.	

Fig. 11. REACTIONS BETWEEN TUMOR 13738b AND TYPES OF MICE EMPLOYED

between the observed and expected ratios, which is apparently not significant and can probably be safely ignored.

The striking exception which places this tumor and 15091a in a group by themselves is the unexpected susceptibility of about one-third of the individuals in the D stock. Instead of the expected 100 per cent negative, we see that here 31.43 per cent of the mice grew tumor 13738b. Similarly there are 12.09 per cent of the MD individuals which were susceptible when all were expected to be negative. The pedigrees of all the exceptional individuals of the D and MD stocks were traced back for ten generations and all of the mice were found to be produced by strict brother-to-sister matings. A possible explanation of the reaction of the D and MD individuals will be taken up after we describe the reactions of the other types of mice which were employed.

The individuals of the F_1 (D $\times$ A), F_1 (MD $\times$ A), and F_1 (N $\times$ A) generations were all susceptible as expected. The N stock animals were all non-susceptible, as they were expected to be. Thus they were different in their reaction from the individuals of the D and MD stocks, which were also expected to be negative.

When we examine the data for the F_2 (D $\times$ A) generation individuals, we see that the observed ratio for 308 mice is significantly different from the expected ratios for both one and two factors, lying about halfway between them. On the other hand, the F_2 (N $\times$ A) data give an observed ratio for 246 mice which is significantly different from the expected ratios for one and for three factors but which differs by only 0.76 times the probable error from the expected ratio for two factors. The need of the presence of two susceptibility factors would readily explain the data obtained when tumor 13738b is transplanted into the F_2 (N $\times$ A) individuals. There is no distortion of the ratios obtained when we consider only the N stock individuals and the (N $\times$ A) generations of hybrids. It has, however, been shown above that there is some ratio-distorting influence possessed by certain of the D stock individuals. This influence is apparently transmitted to at least some of the mice of the F_2 (D $\times$ A) generation. An examination of Fig. 12 shows that when taken by experiments, arranged in chronological order, the observed ratios are evidently fluctuations of the same ratio. Ratios 3 and 7 contain too few animals to make a significant comparison, but all the other ratios show a difference of less than two times the probable error between them. This does not warrant a division of the data into early and late periods

of experimentation. For this reason we cannot assume that a somatic mutation had occurred. Furthermore, contemporary experiments with tumor 13738b in the F_2 ($N \times A$) mice showed no evidence that such a change had taken place.

Experiments	Reactions		Totals	Per Cent Negative
	Positive	Negative		
1. 3N to 6Q	41	19 ± 2.43	60	31.66 ± 4.05
2. 7P	26	18 ± 2.19	44	40.90 ± 4.98
3. 9K	21	5 ± 1.35	26	19.23 ± 5.19
4. 15I	23	13 ± 1.94	36	36.11 ± 5.39
5. 15U	32	15 ± 2.15	47	31.91 ± 4.57
6. 16G	46	19 ± 2.46	65	29.23 ± 3.78
7. 17C	17	13 ± 1.82	30	43.33 ± 6.07
Difference between ratios 1 and 2 = $9.24\% \pm 6.42$ or $1.44 \times \text{P.E.}$ Difference between ratios 1 and 7 = $11.67\% \pm 7.29$ or $1.59 \times \text{P.E.}$ Difference between ratios 2 and 5 = $8.99\% \pm 6.75$ or $1.33 \times \text{P.E.}$ Difference between ratios 2 and 6 = $11.67\% \pm 6.25$ or $1.86 \times \text{P.E.}$ Difference between ratios 3 and 7 = $24.10\% \pm 7.98$ or $3.01 \times \text{P.E.}$ Difference between ratios 5 and 7 = $11.42\% \pm 7.59$ or $1.50 \times \text{P.E.}$ Difference between ratios 6 and 7 = $14.10\% \pm 7.15$ or $1.97 \times \text{P.E.}$				

FIG. 12. REACTIONS OF F_2 ($D \times A$) INDIVIDUALS TO TRANSPLANTS OF 13738b, BY EXPERIMENTS

Experiments	Reactions		Totals	Per Cent Negative
	Positive	Negative		
1. 7Q	6	36 ± 1.79	42	85.71 ± 4.26
2. 7X	25	34 ± 2.55	59	57.62 ± 4.32
3. 8T	10	8 ± 1.42	18	44.44 ± 7.89
4. 9J	7	18 ± 1.51	25	72.00 ± 6.04
5. 15V	9	8 ± 1.35	17	47.06 ± 7.94
6. 19H	7	31 ± 1.61	38	81.58 ± 4.23
Difference between ratios 1 and 2 = $28.09\% \pm 6.06$ or $4.62 \times \text{P.E.}$ Difference between ratios 2 and 6 = $23.96\% \pm 6.00$ or $3.99 \times \text{P.E.}$				

FIG. 13. REACTIONS OF 1BC INDIVIDUALS TO TRANSPLANTS OF 13738b, BY EXPERIMENTS

The results with the 1BC generation individuals bear out the above findings. When the experiments are arranged chronologically, the individuals of the 1BC generation give no evidence of the occurrence of a definite change toward a lower degree of tissue specificity in tumor 13738b (Fig. 13). When we study the total data for reactions of the 1BC individuals (Fig. 11), we find the

observed ratio is significantly different from the expected ratios for one and for three factors. The difference between the observed ratio and the expected ratio for two factors is 2.35 times the probable error. This does not exclude the possibility that two susceptibility factors were needed by the individuals which grow the tumor. The odds against this are about 7.5 : 1 (according to Pearl, 1930). This possibility is not supported by the data from the mice of the F_2 ($D \times A$) generation. On the other hand, the data from the individuals of the F_2 ($N \times A$) generation show clearly that the need of the presence of two susceptibility factors would readily explain the results obtained.

In an attempt to explain the reactions of tumor 13738b several hypotheses have been tried, and one of these is given as a possible explanation which does fit the observed data fairly closely. This, however, is only a working hypothesis. When we analyzed the D stock according to the reactions of its individuals, several points were evident:

(1) Certain D stock individuals, but not all, have a tendency (probably genetic) to encourage the growth of tumor 13738b.

(2) The data of the reactions of the D individuals are not significantly different from a 3 — : 1 + or a one-factor mendelian ratio (Fig. 11).

(3) Susceptibility in the D individuals appears like the homozygous recessive condition (ii) of a dominant inhibitor and allows tumor growth.

(4) The (ii) factor combination apparently operates independently of the dominant susceptibility factors carried by the A stock individuals.

(5) If this hypothesis is true, the D stock is composed of three types of individuals in the proportion of 1 II : 2 Ii : 1 ii, or three non-susceptible animals with the dominant inhibitor to one susceptible animal with the (ii) factor combination.

(6) This 3 : 1 ratio could remain in this proportion indefinitely, for an equal number of I and i gametes would be formed in the population as a whole.

The A stock mice have been shown to carry all the dominant susceptibility factors necessary for the growth of tumor 13738b. If the above hypothesis is true, the segregating hybrid generations of a $D \times A$ cross should show distorted ratios when tested for susceptibility to tumor 13738b. This distortion would be caused by a certain number of individuals which, like the D stock animals,

Gametes of F ₁ (D × A) Individuals	3GHI	3GhI	3gHI	3ghI	GHI	gHi	Ghi	ghi
3GHI	9GGHHII +	9GGHhII +	9GgHHII +	9GgHhII +	3GGHHII +	3GgHHII +	3GGHhII +	3GgHhII +
3GhI	9GGHhII +	9GGhhII -	9GgHhII +	9GghhII -	3GGHhII +	3GgHhII +	3GGhhII -	3GghhII -
3gHI	9GGHHII +	9GgHHII +	9ggHHII -	9ggHhII -	3GgHHII +	3ggHHII -	3GgHhII +	3ggHhII -
3ghI	9GGHhII +	9GghhII -	9GgHhII +	9gghhII -	3GgHhII +	3ggHhII -	3GghhII -	3gghhII -
GHI	3GGHHII +	3GGHhII +	3GgHHII +	3GgHhII +	GGHHII +	GgHHII +	GGHhII +	GgHhII +
gHi	3GgHHII +	3GgHhII +	3ggHHII -	3ggHhII -	GgHHII +	ggHHII +	GgHhII +	ggHhII +
Ghi	3GGHhII +	3GgHhII +	3GgHhII +	3GghhII -	GGHhII +	GgHhII +	GGhhII +	GghhII +
ghi	3GgHhII +	3GghhII -	3ggHhII +	3gghhII -	GgHhII +	ggHhII +	GghhII +	gghhII +

Fig. 14. TYPES OF F₂ (D × A) INDIVIDUALS FORMED WHEN THE HYPOTHETICAL ii FACTOR TAKEN ALONE PRODUCES SUSCEPTIBILITY
Excess susceptible individuals are marked ++.

were susceptible because of the presence of the *ii* factor. This susceptibility would show itself without the aid of susceptibility factors contributed by parent mice of the A stock. Both the F_2 ($D \times A$) and the 1BC generation data do show such distorted ratios. To correct these ratios, the individuals susceptible only because of the factor *ii* should be subtracted from the susceptible group and added to the non-susceptible group. This should be done before the ratios can be analyzed to determine the number of dominant susceptibility factors brought into the cross by the A parents.

Gametes of F_1 ($D \times A$) Individuals	Gametes of D Individuals	
	ghI	ghi
3GHI	3GgHhII	3GgHhIi
	+	+
3GhI	3GgghhII	3GgghhIi
	—	—
3gHI	3ggHhII	3ggHhIi
	—	—
3ghI	3ggghhII	3ggghhIi
	—	—
GHI	GgHhIi	GgHhii
	+	+
gHI	ggHhIi	ggHhii
	—	++
Ghi	GgghhIi	Ggghhii
	—	++
ghi	ggghhIi	ggghhii
	—	++

FIG. 15. TYPES OF 1BC INDIVIDUALS FORMED WHEN THE HYPOTHETICAL *ii* FACTOR TAKEN ALONE PRODUCES SUSCEPTIBILITY
Excess susceptible individuals are marked ++.

We have already seen that more than one and less than three susceptibility factors are probably contributed by the A parents. The data for the 1BC and the F_2 ($N \times A$) reactions point toward the probable need of two factors. Let us assume that these are G and H. To return to our hypothesis, all A stock individuals would be genetically GGHHII and the D stock population would be in the proportion of 1gghhII : 2ggghhIi : 1ggghhii or 3 — : 1 +. The gametes of A stock mice would all be of one kind, GHI. The gametes of D stock mice would be of two kinds in the proportion of 1ghI : 1ghi. Random matings between the individuals of the D stock and the A stock would produce susceptible F_1 ($D \times A$) mice of two kinds, GgHhIi and GgHhii. Random matings be-

Types of Mice	Observed Reactions		Expected Reactions for Number of Mice			Difference between Observed and Expected Ratios	Total Mice
	Ratios	Per Cent Negative	Factors Needed	Expected Ratios	Per Cent Negative		
A	160.00+ : 0.00-	00.00		160.00+ : 0.00-	00.00	00.00%	160
D	26.00+ : 57.00- ± 2.84	68.67 ± 3.42	1 recessive	00.00+ : 83.00-	00.00	31.33%	83
			2 recessive	20.75+ : 62.25- ± 2.66	75.00 ± 3.20	6.33% ± 4.68 or 1.35 × P.E.	
MD				5.19+ : 77.81- ± 1.48	93.75 ± 1.78	25.08% ± 3.85 or 6.51 × P.E.	
F ₁ (D × A)	28.00+ : 100.00- ± 3.15	78.12 ± 2.46		0.00+ : 128.00-	100.00	21.88%	128
F ₁ (MD × A)	101.00+ : 0.00-	00.00		101.00+ : 0.00-	00.00	00.00%	101
ZBC	17.00+ : 0.00-	00.00		17.00+ : 0.00-	00.00	00.00%	17
F ₂ (D × A)	93.00+ : 0.00-	00.00	1 dominant	93.00+ : 0.00-	00.00	00.00%	93
	208.00+ : 104.00- ± 5.61	33.33 ± 1.80	2 dominant	234.00+ : 78.00- ± 5.15	25.00 ± 1.65	8.33% ± 2.44 or 3.41 × P.E.	312
Corrected				175.50+ : 136.50- ± 5.90	43.75 ± 1.89	10.42% ± 2.61 or 3.99 × P.E.	
1BC	198.36+ : 113.64- ± 5.73	36.42 ± 1.83	2 dominant	175.50+ : 136.50- ± 5.90	43.75 ± 1.89	7.33% ± 2.63 or 2.78 × P.E.	
	95.00+ : 135.00- ± 5.03	58.69 ± 2.19	1 dominant	115.00+ : 115.00- ± 5.11	50.00 ± 2.22	8.69% ± 2.93 or 2.96 × P.E.	230
Corrected				57.50+ : 172.50- ± 4.42	75.00 ± 1.92	16.31% ± 2.91 or 5.60 × P.E.	
	69.09+ : 160.91- ± 4.68	69.96 ± 2.03	2 dominant	57.50+ : 172.50- ± 4.42	75.00 ± 1.92	5.04% ± 2.79 or 1.80 × P.E.	

FIG. 16. REACTIONS BETWEEN TUMOR 15091a AND TYPES OF MICE EMPLOYED

tween these F_1 mice would produce the types of F_2 ($D \times A$) animals shown in Fig. 14. Similarly random matings with the D stock mice and the F_1 ($D \times A$) animals would give the types of mice shown in Fig. 15. By this hypothesis 7/151 of the susceptible F_2 ($D \times A$) and 3/11 of the susceptible 1BC individuals would grow tumor 13738b only because they carried the ii factor complex. These would be the excessive susceptible individuals who did not carry both factors G and H in a homozygous dominant or a heterozygous condition.

If we return to the data obtained by transplanting tumor 13738b into individuals of the F_2 ($D \times A$) generation (Fig. 11), and correct the observed ratio for the hypothetical 7/151 excess of susceptible animals, we obtain a ratio which differs by 2.84 times the probable error from the ratio expected when two susceptibility factors are needed. This difference is probably not significant and shows that the hypothesis fits fairly closely for the F_2 ($D \times A$) data. When this hypothesis is tried on the data for the 1BC individuals, we obtain a corrected ratio which differs by only 0.55 times the probable error from the expectation for two susceptibility factors. This difference is so small that the corrected ratio and the two-factor expectation are practically the same. The above hypothesis has been shown to apply quite closely to this tumor and, as will be shown later, it fits even more closely the data for tumor 15091a.

2. The data for the reactions between tumor 15091a and the types of mice receiving it are shown in Fig. 16. We can see at a glance that outside of the D and MD stocks and the F_2 ($D \times A$) and 1BC generations no exceptional individuals were encountered.

The data for the D stock individuals show a difference of 31.33 per cent between the observed and the expected ratios. This is almost identical with the 31.43 per cent difference seen in ratios for tumor 13738b in this same type of mice. There is a difference of only 1.35 times the probable error between the observed ratio and the expected ratio when one recessive susceptibility factor is involved in the growth of tumor 15091a. This is not significant, and the hypothesis given above could readily explain the susceptibility of the D stock mice which grew tumor 15091a.

The data for the individuals of the MD stock show that, instead of all being negative, 21.88 per cent of these animals were susceptible. This is not significantly different from the expected result when one recessive susceptibility factor is involved in the

Tumor	Demonstrated by Individuals of	When the Observed Ratio is Compared with the Expectation for	Difference between the Observed Ratio and Expectation	Probable Number of Factors
1. 13738a	F ₂ (D × A) 294 mice	1 factor 2 factors 3 factors	9.21 × P.E. 1.88 × P.E. 3.20 × P.E.	2
	1BC 205 mice	1 factor 2 factors 3 factors	7.91 × P.E. 0.17 × P.E. 5.02 × P.E.	2
2. 13738b	F ₂ (D × A) 308 mice	1 factor 2 factors 2 factors (when observed ratio is corrected)	3.31 × P.E. 4.06 × P.E. 2.84 × P.E.	2 (when corrected)
	1BC 199 mice	1 factor 2 factors 2 factors (when observed ratio is corrected)	5.45 × P.E. 2.35 × P.E. 0.55 × P.E.	2
3. 13738c	F ₂ (D × A) 361 mice	1 factor 2 factors 3 factors	9.77 × P.E. 1.45 × P.E. 4.21 × P.E.	2
	1BC 186 mice	1 factor 2 factors 3 factors 4 factors	9.79 × P.E. 1.94 × P.E. 2.69 × P.E. 5.77 × P.E.	2 or 3
4. 14059a	F ₂ (D × A) 219 mice	3 factors 4 factors 5 factors 6 factors	4.89 × P.E. 1.45 × P.E. 1.31 × P.E. 3.59 × P.E.	4 or 5
	1BC 116 mice	2 factors 3 factors 4 factors 5 factors 6 factors	6.15 × P.E. 1.43 × P.E. 1.03 × P.E. 2.68 × P.E. 3.71 × P.E.	3 to 5 probably 4
5. 14905a	F ₂ (D × A) 221 mice	3 factors 4 factors 5 factors 6 factors	3.90 × P.E. 0.45 × P.E. 2.32 × P.E. 4.63 × P.E.	4 or 5 probably 4
	1BC 87 mice	2 factors 3 factors 4 factors 5 factors 6 factors 7 factors	5.02 × P.E. 1.87 × P.E. 0.25 × P.E. 1.70 × P.E. 2.62 × P.E. 3.16 × P.E.	3 to 6 probably 4

FIG. 17. PROBABLE NUMBER OF MULTIPLE MENDELIAN SUSCEPTIBILITY FACTORS
NEEDED BY EACH OF THE EIGHT TRANSPLANTED TUMORS FOR
ITS CONTINUED GROWTH (cont. on page 603)

Tumor	Demonstrated by Individuals of	When the Observed Ratio is Compared with the Expectation for	Difference between the Observed Ratio and Expectation	Probable Number of Factors
6. 15091a	F ₂ (D × A) 312 mice	1 factor 2 factors 2 factors (when observed ratio is corrected)	3.41 × P.E. 3.99 × P.E. 2.78 × P.E.	2 (when corrected)
	1BC 230 mice	1 factor 2 factors 2 factors (when observed ratio is corrected)	2.96 × P.E. 5.60 × P.E. 1.80 × P.E.	2 (when corrected)
7. 16189a	F ₂ (D × A) 114 mice	8 factors 9 factors 10 factors 11 factors 12 factors 13 factors	2.96 × P.E. 1.99 × P.E. 1.15 × P.E. 0.42 × P.E. 0.21 × P.E. 0.75 × P.E.	8 to over 13 probably around 12
	1BC 101 mice	? All non-susceptible	?	Probably over 8
8. 17495a undivided data	F ₂ (D × A) 372 mice	1 factor 2 factors	3.63 × P.E. 4.99 × P.E.	?
	1BC 236 mice	1 factor 2 factors	3.88 × P.E. 4.62 × P.E.	?
17495a original	F ₂ (D × A) 128 mice	1 factor 2 factors 3 factors	6.80 × P.E. 0.55 × P.E. 3.64 × P.E.	2
	1BC 87 mice	1 factor 2 factors 3 factors	3.86 × P.E. 1.35 × P.E. 4.54 × P.E.	2
17495a mutant	F ₂ (D × A) 244 mice	1 factor 2 factors	0.66 × P.E. 6.41 × P.E.	1
	1BC 149 mice	1 factor 2 factors	1.99 × P.E. 3.77 × P.E.	1

FIG. 17 (*cont.*). PROBABLE NUMBER OF MULTIPLE MENDELIAN SUSCEPTIBILITY FACTORS NEEDED BY EACH OF THE EIGHT TRANSPLANTED TUMORS FOR ITS CONTINUED GROWTH

growth of the tumor. Since no segregating hybrid generations were available, we cannot say more about the MD stock mice than that they seem to support the above hypothesis that they carry the hypothetical susceptibility factor complex ii.

When the data for the F₂ (D × A) individuals were arranged

Tumors	Individuals of Pure Stocks						Individuals of (D × A) Hybrid Generations											
	A Stock Reactions			D Stock Reactions			F ₁ (D × A) Reactions		ZBC Reactions		F ₂ (D × A) Reactions		IBC Reactions					
	+	-	Total	+	-	Total	+	-	Total	+	-	Total	+	-	Total			
1. 13738a	210	1*	211	0	96	96	106	0	106	103	0	103	150	144	294	52	153	205
2. 13738b	264	1*	265	22	48	70	135	0	135	107	1	108	206	102	308	64	135	199
3. 13738c	155	0	155	0	75	75	121	0	121	87	1	88	190	171	361	36	150	186
4. 14059a	159	1*	160	0	66	66	92	0	92	69	0	69	60	159	219	10	106	116
5. 14905a	206	0	206	0	67	67	105	0	105	91	0	91	67	154	221	6	81	87
6. 15091a	160	0	160	26	57	83	101	0	101	93	0	93	208	104	312	95	135	230
7. 16189a	137	4	141	0	8	8	38	0	38	62	3	65	4	110	114	0	101	101
8. 17495a	211	0	211	1	93	94	117	0	117	67	0	67	249	123	372	91	145	236
Total Transplants	1509			559			815		684		2201		1306					
Exceptional Individuals	*♂Cl 1769(+ 4)			1 (+ 48)			0		2 (+ 3)									

FIG. 18. REACTIONS OF INDIVIDUALS OF THE A STOCK, D STOCK, AND THEIR HYBRID GENERATIONS TO EACH OF THE EIGHT TRANSPLANTED TUMORS
 Susceptible = (+), non-susceptible = (-).

chronologically by experiments, there was no evidence of a change toward less specificity of the tumor tissue. The proportion of negative mice fluctuated around 33 per cent. The total data show that 33.33 per cent of the 312 animals failed to grow tumor 15091a. This is almost identical with the 33.11 per cent of F_2 ($D \times A$) mice who were negative to tumor 13738b. As with tumor 13738b, the observed ratio lies between the expected ratios for one and for two susceptibility factors, but is significantly different from both. If we correct the observed ratio for the hypothetical 7/151 excess of susceptible animals, the corrected ratio differs by 2.78 times the probable error from the expected ratio when two factors are involved in susceptibility. This difference is not significant, and two susceptibility factors are probably contributed by the A parents if this hypothesis is correct.

The data for the 1BC individuals show this same distortion of the observed ratio that makes it definitely different from a two-factor expectation and probably significantly different from the expectation when one factor is involved. When the observed ratio is corrected for the hypothetical 3/11 excess of susceptible animals, the corrected ratio differs by only 1.80 times the probable error from the ratio expected when two dominant susceptibility factors are involved in the growth of tumor 15091a.

If the proposed hypothesis is the correct interpretation of the unexpected reactions of tumors 13738b and 15091a, then it is probable that two different types of susceptibility factors can enter into the growth of these two tumors. There are probably two dominant susceptibility factors contributed by the A parents and a homozygous recessive growth-allowing factor contributed by the D parents.

Fig. 17 shows a summary of all the data given above for the individuals of the F_2 ($D \times A$) and of the 1BC generations. The probable number of dominant susceptibility factors needed for the continued growth of each of these eight transplanted tumors is shown in the right hand column.

Fig. 18 summarizes the reactions of the individuals of the A stock, of the D stock, and of their hybrid generations, to each of these eight transplanted tumors. We have stated above that tumor 16189a did not grow in 100 per cent of the individuals of the susceptible A stock. Because of this, we have four exceptional A stock animals which did not grow 16189a. Outside of these individuals, only one A stock exception was found among 1509

mice. This was male C1 1769, who received transplants of tumors 13738a, 13738b, and 14059a. Except for the three exceptional ZBC individuals which died early and did not grow 16189a, we have only two other exceptional animals out of 684 mice of this generation. All the 815 F_1 ($D \times A$) individuals were susceptible. There were twenty-two D individuals which grew tumor 13738b and twenty-six which grew tumor 15091a. There was only one other D individual which was susceptible to any of the transplants.

II. A Comparative Investigation of the Transplanted Tumors Growing in Different Body Regions of the Same Hosts

This study is an attempt to determine, as nearly as possible, the number of susceptibility factors that would account for the continued growth of all of these transplanted tumors in the same host. Such a factor combination probably exists in a homozygous dominant form in the individuals of the A stock.

The N count of the chromosomes in mice is but twenty, and since there does not appear to be clear evidence of linkage between any of these susceptibility factors there are probably less than twenty susceptibility factors involved. If the factors listed in Fig. 17 were all different, there could be over thirty different factors for susceptibility.

This comparison cannot, however, be applied to tumors 13738b and 15091a, since their distorted ratios prevented the determination of the probable number of necessary factors without the use of an additional hypothesis. Should this hypothesis be true, we could not detect the actual susceptible individuals which were in excess. For this reason we cannot tell if a susceptible individual grew the tumors because of the dominant susceptibility factors introduced by the A parent or because of the hypothetical ii factor.

Tumor 16189a is also of little value in a comparative study of the susceptibility factors involved, for only four F_2 ($D \times A$) individuals and none of the 1BC animals grew this tumor (Fig. 18). As previously shown, the probable number of susceptibility factors necessary for the growth of this tumor could not be determined within several factors (Fig. 17).

This leaves five tumors, 13738a, 13738c, 14059a, 14905a, and 17495a, to be compared. In this study, tumor 13738a has been designated by the Roman numeral I, 13738c by II, 14059a by III, 14905a by IV, 17495a before the mutation by V, and 17495a after the mutation by VI.

A summary of the probable number of susceptibility factors needed by each of these tumors is shown in Fig. 19.

Tumors	Shown by Individuals of	
	F ₂ (D × A) Generation	1BC Generation
I. 13738a	2	2
II. 13738c	2	2 or 3
III. 14059a	4 or 5	3 to 5
IV. 14905a	4 or 5	3 to 6
V. 17495a (original)	2	2
VI. 17495a (mutant)	(1)	(1)
Totals	14 to 16	12 to 18

FIG. 19. PROBABLE NUMBER OF SUSCEPTIBILITY FACTORS NECESSARY FOR THE CONTINUED GROWTH OF THE TRANSPLANTED TUMORS (TAKEN FROM FIG. 17)

From a study of this figure we find that, should these tumors have no susceptibility factors in common, there would be at least fourteen to sixteen factors involved, as shown by the F₂ (D × A) individuals, or twelve to eighteen factors involved, as shown by the 1BC individuals. It is probable that some of these susceptibility factors are common to two or more tumors, and the actual number of factors involved may be less than twelve or fourteen.

The ratio obtained in the comparison of any two of these tumors should not be significantly different from one or more of the theoretical ratios in Fig. 20 unless there is a case of linkage between some of the factors involved.

In the following comparisons the probable numbers of factors needed in common for these tumors are shown in Figs. 21 to 38.

1. Tumor 13738a is compared with tumors 13738c, 14059a, 14905a, and 17495a (before and after its change of specificity).

2. Tumor 13738c is compared with tumors 14059a, 14905a, 17495a (before and after its change).

3. Tumor 14059a is compared with tumor 14905a.

4. Tumor 14905a is compared with tumor 17495a (before its change).

1. Tumor 13738a Compared with Other Tumors:

(a) A comparison of the factor or factors needed in common by tumors 13738a and 13738c:

As shown in Fig. 21, there were sixty-four individuals of the F₂ (D × A) generations which received transplants of both of these tumors. Each of the tumors probably needs two susceptibility

Factors	Susceptibility Factors Needed	Ratio No.	Generation	+B+A	+B-A	-B+A	-B-A	Total Mice
2	A = N	1	F ₂	9	0	3	4	16
	B = NO		1BC	1	0	1	2	4
	A = N	2	F ₂	9	3	3	1	16
	B = O		1BC	1	1	1	1	4
3	A = N	3	F ₂	27	9	21	7	64
	B = OP		1BC	1	1	3	3	8
	A = NO	4	F ₂	27	9	9	19	64
	B = OP		1BC	1	1	1	5	8
	A = N	5	F ₂	27	0	21	16	64
	B = NOP		1BC	1	0	3	4	8
4	A = NO	6	F ₂	27	0	9	28	64
	B = NOP		1BC	1	0	1	6	8
	A = N	7	F ₂	81	0	111	64	256
	B = NOPQ		1BC	1	0	7	8	16
	A = N	8	F ₂	81	27	111	37	256
	B = OPQ		1BC	1	1	7	7	16
	A = NO	9	F ₂	81	0	63	112	256
	B = NOPQ		1BC	1	0	3	12	16
4	A = NO	10	F ₂	81	27	63	85	256
	B = OPQ		1BC	1	1	3	11	16
	A = NO	11	F ₂	81	63	63	49	256
	B = PQ		1BC	1	3	3	9	16
	A = NOP	12	F ₂	81	0	27	148	256
	B = NOPQ		1BC	1	0	1	14	16
	A = NOP	13	F ₂	81	27	27	121	256
	B = OPQ		1BC	1	1	1	13	16

FIG. 20. THEORETICAL RATIOS EXPECTED FOR FROM ONE TO FIVE SUSCEPTIBILITY FACTORS WHEN TWO DIFFERENT TUMORS (A AND B) ARE BOTH TRANSPLANTED INTO ALL OF THE INDIVIDUALS OF LARGE F₂ (D × A) AND 1BC POPULATIONS

factors (Fig. 19) for its continued growth. This is borne out by the fact that the observed ratio (Fig. 21) is not significantly different from the expectation for a 27 : 9 : 9 : 19 ratio (Ratio 2) where both 13738a and 13738c need the presence of two factors. The observed ratio will also fit an 81 : 63 : 27 : 85 ratio where 13738a needs the presence of three and 13738c needs the presence of two susceptibility factors. In either case these two tumors

Factors	Susceptibility Factors Needed	Ratio No.	Generation	+B+A	+B-A	-B+A	-B-A	Total Mice
5	A = N B = NOPQR	14	F ₂ 1BC	243 1	0 0	525 15	256 16	1024 32
	A = N B = OPQR	15	F ₂ 1BC	243 1	81 1	525 15	175 15	1024 32
	A = NO B = NOPQR	16	F ₂ 1BC	243 1	0 0	333 7	448 24	1024 32
	A = NO B = OPQR	17	F ₂ 1BC	243 1	81 1	333 7	367 23	1024 32
	A = NO B = PQR	18	F ₂ 1BC	243 1	189 3	333 7	259 21	1024 32
	A = NOP B = PQR	19	F ₂ 1BC	243 1	189 3	189 3	403 25	1024 32
	A = NOP B = NOPQR	20	F ₂ 1BC	243 1	0 0	189 3	592 28	1024 32
	A = NOPQ B = NOPQR	21	F ₂ 1BC	243 1	0 0	81 1	700 30	1024 32
	A = NOP B = OPQR	22	F ₂ 1BC	243 1	81 1	189 3	511 27	1024 32
	A = NOPQ B = OPQR	23	F ₂ 1BC	243 1	81 1	81 1	619 29	1024 32

FIG. 20. (cont.). THEORETICAL RATIOS EXPECTED FOR FROM ONE TO FIVE SUSCEPTIBILITY FACTORS WHEN TWO DIFFERENT TUMORS (A AND B) ARE BOTH TRANSPLANTED INTO ALL INDIVIDUALS OF LARGE F₂ (D × A) AND 1BC POPULATIONS

probably need the presence of one factor which is common to both of them.⁴

Fig. 22 shows the reactions of sixty-seven 1BC individuals to tumors 13738a and 13738c. Here, as with the F₂ (D × A) animals, the observed ratio is not significantly different from the expectation when each tumor needs the presence of two susceptibility factors.

⁴ Fig. 20 shows the theoretical combination of factors that we could expect to find when two different tumors are transplanted into the same individuals of the segregating hybrid generations. A and B represent the two tumors being compared. Their values given are general theoretical combinations which show the number of factors needed in common by tumors A and B. Therefore, in Figs. 21 to 35 we have given theoretical combinations of susceptibility factors (L, M, N, O, P, Q, R, and S). This is done to express the relationship of the factors involved rather than to show what the actual factor complex really is. This gives the probable number of necessary factors needed in common by two tumors which were transplanted into the same hosts.

Ratios for 64 Mice				
1. Observed F_2 ($D \times A$)				
2. Expected by ratio 4 (Fig. 20) 27:9:9:19				
3. Expected by ratio 10 (Fig. 20) 81:63:27:85				
4. Difference between ratios 1 and 2				
5. Difference between ratios 1 and 3				
By ratio 4 (Fig. 20) = 1 common factor I = MN and II = NP				
By ratio 10 (Fig. 20) = 1 common factor I = LMN and II = NP				
	+ II + I	+ II - I	- II + I	- II - I
	23.00 $\pm$ 2.58	12.00 $\pm$ 2.10	4.00 $\pm$ 1.30	15.00 $\pm$ 2.28
	27.00 $\pm$ 2.66	9.00 $\pm$ 1.87	9.00 $\pm$ 1.87	19.00 $\pm$ 2.46
	20.25 $\pm$ 2.51	15.75 $\pm$ 2.32	6.75 $\pm$ 1.65	21.25 $\pm$ 2.53
	1.08 $\times$ P.E.	1.06 $\times$ P.E.	2.19 $\times$ P.E.	1.19 $\times$ P.E.
	0.76 $\times$ P.E.	1.20 $\times$ P.E.	1.30 $\times$ P.E.	1.82 $\times$ P.E.

FIG. 21. COMPARISON OF THE SUSCEPTIBILITY TO TUMORS 13738a (I) AND 13738c (II) IN THE SAME F_2 ($D \times A$) INDIVIDUALS
I = B and II = A in Fig. 20.

Ratios for 67 Mice				
1. Observed IBC				
2. Expected by ratio 4 (Fig. 20) 1:1:1:5				
3. Difference between ratios 1 and 2				
By ratio 4 (Fig. 20) = 1 common factor I = MN and II = NP				
	+ II + I	+ II - I	- II + I	- II - I
	15.00 $\pm$ 2.30	5.00 $\pm$ 1.45	5.00 $\pm$ 1.45	42.00 $\pm$ 2.66
	8.37 $\pm$ 1.82	8.37 $\pm$ 1.82	8.37 $\pm$ 1.82	41.87 $\pm$ 2.67
	2.25 $\times$ P.E.	1.44 $\times$ P.E.	1.44 $\times$ P.E.	0.03 $\times$ P.E.

FIG. 22. COMPARISON OF THE SUSCEPTIBILITY TO TUMORS 13738a (I) AND 13738c (II) IN THE SAME IBC INDIVIDUALS
I = B and II = A in Fig. 20.

The F_2 ($D \times A$) and the 1BC individuals both indicate that tumors 13738a and 13738c need the presence of two susceptibility factors one of which is needed in common by both tumors.

(b) A comparison of the factor or factors needed in common by tumors 13738a and 14059a.

The observed ratio (Fig. 23) for eighty-nine F_2 ($D \times A$) individuals which received transplants of these two tumors fits closely the expectation for an 81 : 0 : 63 : 112 ratio. This shows that 13738a probably needs the presence of two and 14059a probably needs the presence of four susceptibility factors. The expected ratio of 243 : 0 : 333 : 448 is also quite close to the observed ratio, with no significant difference between them. This expectation shows that 13738a probably needs the presence of two and 14059a probably needs the presence of five susceptibility factors. Both of these interpretations are in agreement with Fig. 19. In either case 14059a needs the presence of the two factors which are needed for the growth of tumor 13738a. Therefore, there probably are two factors that are needed in common by these tumors.

(c) A comparison of the factors needed in common by tumors 13738a and 14905a.

The observed ratio (Fig. 24) for eighty-seven 1BC individuals is not significantly different from the expectation for a 1 : 1 : 1 : 13 ratio. This would mean that 13738a and 14905a each probably needs the presence of three susceptibility factors and that they would need two of these in common.

There was one animal in the observed ratio which grew 14905a but failed to grow 13738a. It may be that this one individual is an exception and should have grown both 13738a (I) and 14905a (IV). The number of individuals is really too small for a conclusive analysis considering the number of factors that are probably involved. Should this one animal be an exception, then the expectation for a 1 : 0 : 1 : 6 ratio is not significantly different from the observed ratio. However, the expectation for a 1 : 0 : 1 : 14 ratio is much closer and differs but little from the observed ratio. Of these two expectations, the former indicates that these two tumors need two susceptibility factors in common and the latter that they need three susceptibility factors in common.

The observed ratio of 5 : 1 : 7 : 74 (Ratio 1, Fig. 24) is probably correct and, according to it 13,738a and 14905a would each need three susceptibility factors. Two of these factors would be needed in common by both tumors.

Ratios for 89 Mice					
1. Observed F_2 ($D \times A$)					
2. Expected by ratio 9 (Fig. 20) 81:0:63:112					
3. Expected by ratio 16 (Fig. 20) 243:0:333:448					
4. Difference between ratios 1 and 2					
5. Difference between ratios 1 and 3					
	+ III + I	+ III - I	- III + I	- III - I	
	24.00 $\pm$ 2.82	0	22.00 $\pm$ 2.74	43.00 $\pm$ 3.17	
	28.15 $\pm$ 2.95	0	21.89 $\pm$ 2.74	38.93 $\pm$ 3.15	
	21.02 $\pm$ 2.70	0	28.94 $\pm$ 2.98	38.94 $\pm$ 3.15	
	1.01 $\times$ P.E.	0	0.02 $\times$ P.E.	0.91 $\times$ P.E.	
	0.76 $\times$ P.E.	0	1.71 $\times$ P.E.	0.91 $\times$ P.E.	
By ratio 9 (Fig. 20) = 2 common factors I = MN and III = MNQR					
By ratio 16 (Fig. 20) = 2 common factors I = MN and III = LMNQR					

FIG. 23. COMPARISON OF THE SUSCEPTIBILITY TO TUMORS 13738a (I) AND 14059a (III) IN THE SAME F_2 ($D \times A$) INDIVIDUALS
I = A and III = B in Fig. 20.

Ratios for 87 Mice					
1. Observed IBC					
2. Expected by ratio 13 (Fig. 20) 1:1:1:13					
3. Difference between ratios 1 and 2					
4. Observed if 1 exception occurred					
5. Expected by ratio 6 (Fig. 20) 1:0:1:6					
6. Expected by ratio 12 (Fig. 20) 1:0:1:14					
7. Difference between ratios 4 and 5					
8. Difference between ratios 4 and 6					
	+ IV + I	+ IV - I	- IV + I	- IV - I	
	5.00 $\pm$ 1.46	1.00 $\pm$ 0.66	7.00 $\pm$ 1.70	74.00 $\pm$ 2.24	
	5.43 $\pm$ 1.52	5.43 $\pm$ 1.52	5.43 $\pm$ 1.52	70.69 $\pm$ 2.45	
	0.20 $\times$ P.E.	2.68 $\times$ P.E.	0.68 $\times$ P.E.	1.00 $\times$ P.E.	
	6.00 $\pm$ 1.59	0	7.00 $\pm$ 1.70	74.00 $\pm$ 2.24	
	10.87 $\pm$ 2.08	0	10.87 $\pm$ 2.08	65.25 $\pm$ 2.71	
	5.43 $\pm$ 1.52	0	5.43 $\pm$ 1.52	76.12 $\pm$ 2.07	
	1.86 $\times$ P.E.	0	1.44 $\times$ P.E.	2.49 $\times$ P.E.	
	0.25 $\times$ P.E.	0	0.68 $\times$ P.E.	0.69 $\times$ P.E.	
By ratio 6 (Fig. 20) = 2 common factors I = MN and IV = MNO					
By ratio 12 (Fig. 20) = 3 common factors I = LMN and IV = LMNO					
By ratio 13 (Fig. 20) = 2 common factors I = LMN and IV = LNO					

FIG. 24. COMPARISON OF THE SUSCEPTIBILITY TO TUMORS 13738a (I) AND 14905a (IV) IN THE SAME IBC INDIVIDUALS
I = A and IV = B in Fig. 20.

(d) A comparison of the factors needed in common by tumors 13738a and 17495a before its change of specificity.

The observed ratio for the F_2 ($D \times A$) individuals is made up of only forty-four animals (Fig. 25) and is not as valuable as the data obtained from the reactions of the eighty-seven 1BC individuals (Fig. 26). The expectation for a 1 : 1 : 0 : 6 ratio is not significantly different from the observed ratio for the reactions of the 1BC individuals (Ratio 3, Fig. 26). This expectation could well explain the data obtained and indicates that tumor 13738a needs the presence of three and the original tumor 17495a needs the presence of two susceptibility factors. Furthermore, it is probable that both of these necessary susceptibility factors are needed in common by both tumors.

(e) A comparison of the factors needed in common by tumors 13738a and 17495a after its change of specificity.

An inspection of Fig. 27 shows that there is no significant difference between the observed ratio and the expectation for a 9 : 3 : 0 : 4 ratio. This expectation indicates that tumor 13738a probably needs the presence of two susceptibility factors and the mutant tumor 17495a probably needs the presence of but one factor for susceptibility. Furthermore, this expectation indicates that the one factor necessary for the mutant tumor 17495a is also needed by tumor 13738a.

The expectation for a 27 : 9 : 0 : 28 ratio is also near enough to the observed ratio so that the difference between the two ratios is not statistically significant. However, this expectation is not quite as near to the observed ratio as the first mentioned expectation (9 : 3 : 0 : 4). This second expectation ratio indicates that 13738a needs the presence of three susceptibility factors, which is not the same as the findings shown in Fig. 17. This ratio also indicates that the mutant tumor needs the presence of two susceptibility factors, which is not substantiated by the more extensive data shown in Fig. 17.

The data for the reactions of the 1BC individuals (Fig. 28) are the results of observations on only thirty-six mice and are, therefore, not as valuable as the data collected for the F_2 ($D \times A$) individuals.

The above analyses show that tumor 13738a probably needs the presence of two susceptibility factors, one of which is the same factor that is necessary for the growth of the mutant tumor 17495a.

Ratios for 44 Mice		+ V	+ I	+ V	- I	- V	+ I	- V	- I
1. Observed F_2 ($D \times A$)		12.00	$\pm$ 1.99	14.00	$\pm$ 2.08	3.00	$\pm$ 1.13	15.00	$\pm$ 2.12
2. Expected by ratio 10 (Fig. 20) 81:63:27:85		13.92	$\pm$ 2.08	10.83	$\pm$ 1.92	4.64	$\pm$ 1.37	14.61	$\pm$ 2.10
3. Expected by ratio 18 (Fig. 20) 243:333:189:259		10.44	$\pm$ 1.90	14.30	$\pm$ 2.09	8.12	$\pm$ 1.73	11.13	$\pm$ 1.94
4. Expected by ratio 19 (Fig. 20) 243:189:189:403		10.44	$\pm$ 1.90	8.12	$\pm$ 1.73	8.12	$\pm$ 1.73	17.31	$\pm$ 2.18
5. Difference between ratios 1 and 2		0.66 $\times$ P.E.		1.12 $\times$ P.E.		0.92 $\times$ P.E.		0.13 $\times$ P.E.	
6. Difference between ratios 1 and 3		0.56 $\times$ P.E.		0.10 $\times$ P.E.		2.48 $\times$ P.E.		1.34 $\times$ P.E.	
7. Difference between ratios 1 and 4		0.56 $\times$ P.E.		2.17 $\times$ P.E.		2.48 $\times$ P.E.		0.75 $\times$ P.E.	
By ratio 10 (Fig. 20) = 1 common factor		I = LMN and V = LS, MS, or NS							
By ratio 18 (Fig. 20) = 0 common factor		I = LMN and V = RS							
By ratio 19 (Fig. 20) = 1 common factor		I = LMN and V = LRS, MRS, or NRS							

Fig. 25. COMPARISON OF THE SUSCEPTIBILITY TO TUMORS 13738a (I) AND 17495a ORIGINAL (V) IN THE SAME F_2 ($D \times A$) INDIVIDUALS
I = B and V = A in Fig. 20.

Ratios for 87 Mice		+ V	+ I	+ V	- I	- V	+ I	- V	- I
1. Observed 1BC		12.00 $\pm$ 2.16		15.00 $\pm$ 2.37		0		60.00 $\pm$ 2.90	
2. Expected by ratio 6 (Fig. 20) 1:1:0:6		10.87 $\pm$ 2.08		10.87 $\pm$ 2.08		0		65.25 $\pm$ 2.72	
3. Difference between ratios 1 and 2		0.37 $\times$ P.E.		1.30 $\times$ P.E.		0		1.32 $\times$ P.E.	
By ratio 6 (Fig. 20) = 2 common factors I = LMN and V = LM or LN									

Fig. 26. COMPARISON OF THE SUSCEPTIBILITY TO TUMORS 13738a (I) AND 17495a ORIGINAL (V) IN THE SAME 1BC INDIVIDUALS
I = B and V = A in Fig. 20.

Ratios for 102 Mice		+ VI	+ I	+ VI	- I	- VI	+ I	- VI	- I
1. Observed F_2 ($D \times A$)		55.00 $\pm$ 3.39		12.00 $\pm$ 2.19		0		35.00 $\pm$ 3.23	
2. Expected by ratio 1 (Fig. 20) 9 : 3 : 0 : 4		57.37 $\pm$ 3.38		19.12 $\pm$ 2.65		0		25.50 $\pm$ 2.95	
3. Expected by ratio 6 (Fig. 20) 27 : 9 : 0 : 28		43.03 $\pm$ 3.36		14.34 $\pm$ 2.36		0		44.62 $\pm$ 3.38	
4. Difference between ratios 1 and 2		0.49 $\times$ P.E.		2.07 $\times$ P.E.		0		2.17 $\times$ P.E.	
5. Difference between ratios 1 and 3		2.50 $\times$ P.E.		0.72 $\times$ P.E.		0		2.05 $\times$ P.E.	
By ratio 1 (Fig. 20) = 1 common factor		I = MN and VI = M or N							
By ratio 6 (Fig. 20) = 2 common factors		I = LMN and VI = LM or LN							

Fig. 27. COMPARISON OF SUSCEPTIBILITY TO TUMORS 13738a (I) AND 17495a MUTANT (VI) IN THE SAME F_2 ($D \times A$) INDIVIDUALS
I = B and VI = A in Fig. 20.

Ratios for 36 Mice									
1. Observed 1BC									
2. Expected by ratio 2 (Fig. 20) 1:1:1:1									
3. Difference between ratios 1 and 2									
		+ VI	+ I	+ VI	- I	- VI	+ I	- VI	- I
		14.00 $\pm$ 1.97		6.00 $\pm$ 1.50		3.00 $\pm$ 1.11		13.00 $\pm$ 1.94	
		9.00 $\pm$ 1.74		9.00 $\pm$ 1.74		9.00 $\pm$ 1.74		9.00 $\pm$ 1.74	
		1.90 $\times$ P.E.		1.30 $\times$ P.E.		2.89 $\times$ P.E.		1.53 $\times$ P.E.	
By ratio 2 (Fig. 20) = No common factors I = M or N and VI = N or S									

Fig. 28. COMPARISON OF SUSCEPTIBILITY TO TUMORS 13738a (I) AND 17495a MUTANT (VI) IN THE SAME 1BC INDIVIDUALS
I = B and VI = A in Fig. 20.

Ratios for 101 Mice		+ III	+ II	+ III	- II	- III	+ II	- III	- II
1. Observed 1BC		5.00 ± 1.47		5.00 ± 1.47		5.00 ± 1.47		86.00 ± 2.41	
2. Expected by ratio 19 (Fig. 20) 1 : 3 : 3 : 25		3.19 ± 1.18		9.56 ± 1.95		9.56 ± 1.95		78.70 ± 2.77	
3. Difference between ratios 1 and 2		0.96 × P.E.		1.86 × P.E.		1.86 × P.E.		1.98 × P.E.	
By ratio 19 (Fig. 20) = 1 common factor II = NOP and III = NQR									

FIG. 29. COMPARISON OF SUSCEPTIBILITY TO TUMORS 13738c (II) AND 14059a (III) IN THE SAME 1BC INDIVIDUALS
II = A and III = B in Fig. 20.

Ratios for 120 Mice		+ IV	+ II	+ IV	- II	- IV	+ II	- IV	- II
1. Observed F_2 ($D \times A$)		30.00	± 3.19	9.00	± 1.94	26.00	± 3.05	55.00	± 3.68
2. Expected by ratio 17 (Fig. 20) 243 : 81 : 333 : 367		28.45	± 3.13	9.48	± 1.99	39.02	± 3.46	43.01	± 3.54
3. Expected by ratio 22 (Fig. 20) 243 : 81 : 189 : 511		28.45	± 3.13	9.48	± 1.99	22.13	± 2.86	59.83	± 3.69
4. Difference between ratios 1 and 2		0.34 $\times$ P.E.		0.17 $\times$ P.E.		2.82 $\times$ P.E.		2.35 $\times$ P.E.	
5. Difference between ratios 1 and 3		0.34 $\times$ P.E.		0.17 $\times$ P.E.		0.92 $\times$ P.E.		0.92 $\times$ P.E.	
By ratio 17 (Fig. 20) = 1 common factor								II = NP and IV = OPQR	
By ratio 22 (Fig. 20) = 2 common factors								II = NOP and IV = OPQR	

FIG. 30. COMPARISON OF SUSCEPTIBILITY TO TUMORS 13738c (II) AND 14905a (IV) IN THE SAME F₂ (D × A) INDIVIDUALS
II = A and IV = B in Fig. 20.

2. Tumor 13738c Compared with Other Tumors:

(a) A comparison of the factor or factors needed in common by tumors 13738c and 14059a.

An inspection of Fig. 29 shows that the observed ratio is quite close to the expectation for a 1 : 3 : 3 : 25 ratio for 101 individuals of the 1BC generation. There is no significant difference between these two ratios. This would indicate that both tumor 13738c and tumor 14059a probably need the presence of three susceptibility factors. There is one factor which is probably needed in common by these two tumors.

(b) A comparison of the factor or factors needed in common by tumors 13738c and 14905a.

An inspection of Fig. 30 shows that results obtained with 120 individuals of the F_2 ($D \times A$) generation are not significantly different from two independent ratios expected for this number of mice.

The expectation for a 243 : 81 : 333 : 367 ratio indicates that 13738c needs the presence of two and 14905a needs the presence of four susceptibility factors. This is substantiated by the data shown in Fig. 17. Probably only one of these susceptibility factors is needed in common by these two tumors.

The expectation for a 243 : 81 : 189 : 511 ratio is somewhat closer to the observed ratio, but it indicates that tumor 13738c needs the presence of three susceptibility factors in the F_2 ($D \times A$) individuals. By this same expectation, tumor 14905a probably needs four susceptibility factors, two of which are needed in common by tumors 13738c and 14905a.

It is probable that the first interpretation is more nearly correct.

(c) A comparison of the factor or factors needed in common by tumor 13738c and tumor 17495a before its change of specificity.

This is shown in Fig. 31, which gives the reactions of 105 F_2 ($D \times A$) individuals to transplants of these two tumors. The observed ratio is not significantly different from the expectation for a 27 : 9 : 9 : 19 ratio. This indicates that tumor 13738c and the original tumor 17495a each probably needs two susceptibility factors and that one of these factors appears to be needed in common by both tumors.

(d) A comparison of the factor or factors needed in common by tumor 13738c and tumor 17495a after its change of specificity.

An inspection of Fig. 32 shows that the observed ratio is not significantly different from the expectation for a 9 : 3 : 0 : 4 ratio for 100 individuals of the F_2 ($D \times A$) generation.

Ratios for 105 Mice					
1. Observed F_2 ($D \times A$)					
2. Expected by ratio 4 (Fig. 20) 27:9:9:19					
3. Difference between ratios 1 and 2					
By ratio 4 (Fig. 20) = 1 common factor II = NP and V = NO					
	+ V + II	+ V - II	- V + II	- V - II	
	50.00 $\pm$ 3.44	8.00 $\pm$ 1.73	7.00 $\pm$ 1.72	40.00 $\pm$ 3.95	
	44.28 $\pm$ 3.41	14.76 $\pm$ 2.40	14.76 $\pm$ 2.40	31.15 $\pm$ 2.15	
	1.18 $\times$ P.E.	2.25 $\times$ P.E.	2.63 $\times$ P.E.	1.92 $\times$ P.E.	

FIG. 31. COMPARISON OF SUSCEPTIBILITY TO TUMORS 13738c (II) AND 17495a ORIGINAL (V) IN THE SAME F_2 ($D \times A$) INDIVIDUALS
II = B and V = A in Fig. 20.

Ratios for 100 Mice					
1. Observed F_2 ($D \times A$)					
2. Expected by ratio 1 (Fig. 20) 9:3:0:4					
3. Difference between ratios 1 and 2					
By ratio 1 (Fig. 20) = 1 common factor II = NP and VI = N or P					
	+ VI + II	+ VI - II	- VI + II	- VI - II	
	68.00 $\pm$ 3.14	9.00 $\pm$ 1.93	0	23.00 $\pm$ 2.83	
	56.25 $\pm$ 3.34	18.75 $\pm$ 2.63	0	25.00 $\pm$ 2.92	
	2.56 $\times$ P.E.	2.99 $\times$ P.E.	0	0.49 $\times$ P.E.	

FIG. 32. COMPARISON OF SUSCEPTIBILITY TO TUMORS 13738c (II) AND 17495a MUTANT (VI) IN THE SAME F_2 ($D \times A$) INDIVIDUALS
II = B and VI = A in Fig. 20.

The difference between these ratios approaches significance in only one case. However, the deviation is not quite three times the probable error.

This expectation probably fits the observed ratio and indicates that tumor 13738c needs the presence of two susceptibility factors, while the mutant of tumor 17495a needs the presence of but one susceptibility factor. This assumption is substantiated by the data shown in Fig. 17. Furthermore, the one susceptibility factor apparently needed by the mutant of tumor 17495a is also probably needed by tumor 13738c.

3. *Tumor 14095a Compared with Tumor 14905a to Show the Factors Needed in Common:* An inspection of Fig. 33 shows that too few individuals are included to make a good comparison of these tumors. This is especially true since four or five susceptibility factors are probably needed for the growth of each of these tumors (Fig. 17).

The observed ratio may or may not be significantly different from the expectation for a 243 : 81 : 81 : 619 ratio. It appears that three susceptibility factors may be needed in common by these two tumors. However, we cannot estimate with much accuracy how many factors really are needed in common by tumors 14059a and 14905a.

4. *Tumor 14905a Compared with Tumor 17495a Before Its Change of Specificity:* An inspection of Fig. 34 shows that the data for the reactions of the F_2 ($D \times A$) individuals to these two tumors do not include a large number of animals. Ratios 2 and 4, however, do show expectations that differ but little from the observed ratio.

Fig. 35 shows the reactions of eighty-seven individuals of the 1BC generation to these same two tumors.

With both the F_2 ($D \times A$) and the 1BC individuals we get data which fit very closely the expectations when 14905a needs the presence of four or five susceptibility factors and the unchanged tumor 17495a needs the presence of two susceptibility factors. This agrees with the data in Fig. 17. These expectations also indicate that tumor 14905a needs the presence of the same two susceptibility factors that are needed by tumor 17495a before its changed specificity. There probably are two factors needed in common by these two tumors.

We have seen that susceptibility to tumor 13738a probably depends upon two susceptibility factors (Figs. 19, 21, 22, 23, and 24)

Ratios for 58 Mice		+ IV	+ III	+ IV	- III	- IV	+ III	- IV	- III
1. Observed F_2 ($D \times A$)		7.00 $\pm$ 1.67		2.00 $\pm$ 0.93		4.00 $\pm$ 1.29		45.00 $\pm$ 2.14	
2. Expected by ratio 23 (Fig. 20) 243 : 81 : 81 : 619		13.76 $\pm$ 2.18		4.58 $\pm$ 1.38		4.58 $\pm$ 1.38		35.06 $\pm$ 2.51	
3. Observed if 2 exceptions occurred		9.00 $\pm$ 1.85		0		4.00 $\pm$ 1.29		45.00 $\pm$ 2.14	
4. Expected by ratio 21 (Fig. 20) 243 : 0 : 81 : 700		13.76 $\pm$ 2.18		0		4.58 $\pm$ 1.38		39.64 $\pm$ 2.38	
5. Difference between ratios 1 and 2		2.46 $\times$ P.E.		1.55 $\times$ P.E.		0.30 $\times$ P.E.		3.02 $\times$ P.E.	
6. Difference between ratios 3 and 4		1.66 $\times$ P.E.		0		0.30 $\times$ P.E.		1.53 $\times$ P.E.	
By ratio 23 (Fig. 20) = 3 common factors		III = OPQR and IV = NOPQ							
By ratio 21 (Fig. 20) = 4 common factors		III = OPQR and IV = NOPQR							

FIG. 33. COMPARISON OF SUSCEPTIBILITY TO TUMORS 14059a (III) AND 14905a (IV) IN THE SAME F_2 ($D \times A$) INDIVIDUALS
 III = A and IV = B in Fig. 20.

Ratios for 46 Mice		+ IV	+ V	+ IV	- V	- IV	+ V	- IV	- V
1. Observed F_2 ($D \times A$)		9.00 $\pm$ 1.81		0		19.00 $\pm$ 2.24		18.00 $\pm$ 2.23	
2. Expected by ratio 9 (Fig. 20) 81 : 0 : 63 : 112		14.54 $\pm$ 2.12		0		11.31 $\pm$ 1.95		20.12 $\pm$ 2.26	
3. Expected by ratio 14 (Fig. 20) 243 : 0 : 525 : 256		10.91 $\pm$ 1.94		0		23.58 $\pm$ 2.28		11.50 $\pm$ 1.95	
4. Expected by ratio 16 (Fig. 20) 243 : 0 : 333 : 448		10.91 $\pm$ 1.94		0		14.95 $\pm$ 2.13		20.12 $\pm$ 2.26	
5. Difference between ratios 1 and 2		1.99 $\times$ P.E.		0		2.59 $\times$ P.E.		0.66 $\times$ P.E.	
6. Difference between ratios 1 and 3		0.72 $\times$ P.E.		0		1.43 $\times$ P.E.		2.19 $\times$ P.E.	
7. Difference between ratios 1 and 4		0.72 $\times$ P.E.		0		1.31 $\times$ P.E.		0.66 $\times$ P.E.	
By ratio 9 (Fig. 20) = 2 common factors		IV = NOPQ and V = NO							
By ratio 14 (Fig. 20) = 1 common factor		IV = NOPQR and V = N or O							
By ratio 16 (Fig. 20) = 2 common factors		IV = NOPQR and V = NO							

FIG. 34. COMPARISON OF SUSCEPTIBILITY TO TUMORS 14905a (IV) AND 17495a ORIGINAL (V) IN THE SAME F_2 ($D \times A$) INDIVIDUALS
 IV = B and V = A in Fig. 20.

or possibly three susceptibility factors (Figs. 21, 24, and 26). These can be called factors (L), M, and N. Another tumor, 13738c, which is probably dependent upon two (or three) susceptibility factors (Figs. 19, 21, 22, 29, 30, 31, and 32) needs the presence of one of these same factors that are needed by tumor 13738a. We can assume that the factor N is the one needed in common by these two tumors. Another factor, which we may call P, presumably must also be present with the factor N in the animals which are susceptible to tumor 13738c. We can therefore assume that an animal possessing these theoretical factors (L), M, N, and P in a heterozygous or a homozygous dominant condition would be susceptible to both tumors 13738a and 13738c. The factor complex (L) MN would allow the growth of tumor 13738a and the combination NP would by this hypothesis allow the growth of tumor 13738c.

We find that after tumor 17495a mutated, the assumption that only one susceptibility factor was needed would readily explain the data obtained (Figs. 17, 27, and 32). Furthermore, we find that tumors 13738a and 13738c probably need this same susceptibility factor which is needed by the mutant tumor 17495a. This apparently would have to be susceptibility factor N, for this is the one factor which appears to be needed in common by tumors 13738a and 13738c. These above assumptions are quite strongly supported by the data obtained.

If the susceptibility factor N is needed by tumor 17495a after its change of specificity, then it must also have been needed before the mutation occurred. There is quite strong evidence then that the early condition of tumor 17495a needed the presence of susceptibility factor N and one other factor (Figs. 19 and 26). There is also quite good evidence that this second susceptibility factor is needed in common with tumor 13738a; it may be the susceptibility factor L.

By this hypothesis the individuals of the A stock would all carry the factor complex LLMMNNPP and would all be susceptible to tumors 13738a, 13738c, and 17495a. The presence of these four susceptibility factors may account for the growth of these tumors.

These comparisons have divided the data to such an extent that we cannot prove conclusively what susceptibility factors are needed but there are strong indications that the above assumptions are correct.

Ratios for 87 Mice		+ IV	+ V	+ IV	- V	- IV	+ V	- IV	- V
1. Observed 1BC		6.00	± 1.59				21.00	± 2.69	60.00 ± 2.90
2. Expected by ratio 9 (Fig. 20) 1:0:3:12		5.43	± 1.51		0		16.31	± 2.45	65.25 ± 2.72
3. Expected by ratio 16 (Fig. 20) 1:0:7:24		2.72	± 1.17		0		19.03	± 2.59	65.25 ± 2.72
4. Difference between ratios 1 and 2		0.25	$\times$ P.E.		0		1.29	$\times$ P.E.	1.32 $\times$ P.E.
5. Difference between ratios 1 and 3		1.66	$\times$ P.E.		0		0.52	$\times$ P.E.	1.32 $\times$ P.E.
By ratio 9 (Fig. 20) = 2 common factors		IV = NOPQ and V = NO							
By ratio 16 (Fig. 20) = 2 common factors		IV = NOPQR and V = NO							

Fig. 35. COMPARISON OF SUSCEPTIBILITY TO TUMORS 14905a (IV) AND 17495a ORIGINAL (V) IN THE SAME 1BC INDIVIDUALS
IV = B and V = A in Fig. 20.

Tumor 14059a Compared with	Tumor 14059a (III)	
	Theoretical Factor Combinations	Probable Factor Combinations
I. 13738a in 89 F ₂ (D × A) 2 factors in common	MN-- MN---	MN---
II. 13738c in 101 1BC 1 factor in common	N-- O-- P--	N--
IV. 14905a in 58 F ₂ (D × A) 3 factors in common	LM-- LN-- MN--	MN--
Probable factor combination is MN--		

Tumor 14905a Compared with	Tumor 14905a (IV)	
	Theoretical Factor Combinations	Probable Factor Combinations
I. 13738a in 87 1BC 2 factors in common	LM- MN- LN-	LN-
II. 13738c in 120 F ₂ (D × A) 2 factors in common	NO-- OP-- NP--	NO--
III. 14059a in 58 F ₂ (D × A) 3 factors in common	LN-- MN-- LM--	LN--
V. 17495a original in 46 F ₂ (D × A) and in 87 1BC 2 factors in common	LN-- LN---	LN---
Probable factor combination is LNO--		

FIGS. 36 AND 37. THEORETICAL SUSCEPTIBILITY FACTOR COMPLEXES NECESSARY FOR THE CONTINUED GROWTH OF TUMOR 14059a AND TUMOR 14905a
- indicates unidentified factors.

Tumors 14059a and 14905a need the presence of too many factors (Fig. 19) to allow very definite conclusions to be drawn from the data obtained. As shown in Figs. 36 and 37, there is some evidence that the same susceptibility factor N is also needed in common by both these tumors. Beyond this we cannot say more than that there is an indication that tumor 14059a needs the

presence of a factor complex containing the susceptibility factors M and N along with two other susceptibility factors (Fig. 36). Similarly, there is an indication that tumor 14905a needs the presence of a factor complex of four or five susceptibility factors, three of which may be the susceptibility factors L, N, and O (Fig. 37).

It is possible that susceptibility factors L, M, N, O, P, and two or three others will account for the susceptibility of the A stock individuals to tumors 13738a, 13738c, 17495a, 14059a and 14905a. This may reduce the necessary number of susceptibility factors for these five tumors from twelve or fourteen to a possible seven or eight (Fig. 38).

Tumors		Possible Factor Complex Needed for Susceptibility
I.	13738a.....	MN or LMN
II.	13738c.....	NP or NOP
III.	14059a.....	MN(O? or L?)-
IV.	14905a.....	LNO(M?)-
V.	17495a.....	LN
VI.	17495a	
	Mutant.....	N

FIG. 38. THEORETICAL SUSCEPTIBILITY FACTOR COMPLEXES WHICH COULD FIT THE OBSERVED DATA FOR THESE TUMORS
- = unidentified factors.

SUMMARY

Four main points were taken up in this experiment. The findings were as follows:

1. *Are the spontaneous mammary gland tumors arising in females of this inbred A line histologically alike?* The tumors included in this problem all appear to be the same type of carcinoma. While they vary somewhat in proportion of stroma and parenchyma, this variation appears to be no greater than the variations which may be encountered in different regions within a single nodule of carcinoma of the mammary gland.

2. *Is the mechanism of transplantation inheritance the same in all cases?*

(a) *Does it involve the same number of susceptibility factors for each tumor used?* The number of susceptibility factors required for each tumor is not the same. The probable number of susceptibility factors needed by tumor 13738a is two; by 13738b, two; by 13738c, two or three, probably two; by 14059a, three to five, probably four; by 14905a, three to six, probably four; by 15091a, two; by 16189a, eight to thirteen or more; and by 17495a,

two at the outset but one in the latter part of the series of experiments. The number of susceptibility factors needed varies with the tumors used for transplantation. Depending upon the different tumors the presence of one, two, three, four, five, six, or eight to thirteen susceptibility factors is necessary in the mice which will grow these transplants.

Each of these eight tumors was transplanted into the individuals of three different stocks of mice and five different types of their hybrids. Tumor 13738b was transplanted into animals of three additional generations. The reactions of these tumors in the different generations of mice are shown in Figures 4-18.

(b) *Are the factors involved in the susceptibility to the different tumors identical in any or all cases?* Tumors 13738b, 15091a, and 16189a gave results which could not be used in an analysis to establish the identity of the susceptibility factors involved. They could not be readily compared with other tumors transplanted into the same hosts. Tumors 13738b and 15091a gave distorted ratios in the segregating hybrid generations, and tumor 16189a required the presence of too many susceptibility factors—thus preventing a comparative study of the susceptibility factors involved in the growth of these tumors.

Tumors 13738a, 13738c, 14059a, 14905a, and 17495a are compared in an attempt to identify the susceptibility factors necessary for the growth of each of them. There is quite a strong indication that a susceptibility factor, which we have called N, is needed in common by these five tumors. There is a further indication that we may be able to identify five of the susceptibility factors which are necessary for the growth of these five tumors. There are two or three more of these factors which we have not identified, nor can we hope to identify with the available data. However, the susceptibility factors needed for these five tumors can probably be reduced from twelve or fourteen factors to seven or eight. The susceptibility factor N is the only one which appears to be needed in common by tumors 13738a, 13738c, 14059a, 14905a, and 17495a.

(c) *Are the same susceptibility factors always necessary for the continued growth of any particular tumor?* The susceptibility factors carried by the A individuals are sufficient to cause the growth of each of these tumors from individuals of the A stock. They are introduced into the hybrids by the A parent, and the individuals of the segregating hybrid generation must have the proper factor complex to be susceptible to a specific tumor. This applies

for each of these eight tumors. In addition to this, certain individuals of the D stock are able to grow tumors 13738b and 15091a without the aid of the susceptibility factors carried by the A individuals. This is also true of certain of the MD individuals with both tumors but not of the N stock animals with tumor 13738b. The segregating hybrids of the ($D \times A$) cross gave distorted ratios for these two tumors. It appears as though the individuals of the D stock carried a dominant factor (I) which inhibits the growth of these two tumors. Certain of the D individuals and their segregating hybrids react as though the homozygous recessive form of this hypothetical inhibitor (i) was carried by them. This factorial complex allows the growth of tumors 13738b and 15091a without the aid of the susceptibility factors from the A individuals.

3. *Is there a definite relationship between the histology of these transplanted tumors and the number of mendelian units underlying susceptibility to them?* No, there is not, for there is seen to be no real difference in the histology of these eight tumors; but they do differ from each other in the number of susceptibility factors that must be present in the animals which grow these tumors. One to thirteen or more susceptibility factors are apparently needed, depending upon which of these eight tumors are being transplanted.

4. *Does the nearness of the pedigree relationship of the individuals giving rise to these transplanted tumors tend to simplify the analysis of the number and identity of the susceptibility factors involved in the growth of each tumor?* No, for even the three tumors from female 13738 differed from each other. Tumors 13738a and 13738c were both two-factor tumors, but they had only one susceptibility factor which was needed by both of them. Tumor 13738b grew in some of the D individuals, while tumors 13738a and 13738c did not. However, tumor 15091a reacted similarly to tumor 13738b when transplanted into D animals (see pedigree in Fig. 2).

The three tumors from female 13738 each needed the presence of two susceptibility factors, but two daughters of female 13738 gave rise to tumors which needed the presence of other susceptibility factors. One daughter, 14905, gave rise to a tumor which probably needs four factors for susceptibility; while another daughter, 16189, gave rise to a tumor which needs eight to thirteen susceptibility factors. These cannot be identified with the numbers available. The nearness of pedigree relationship does not seem to influence the number of susceptibility factors involved in

the growth of these transplanted tumors, but it may influence the identity of these factors. In the comparative study of the above tumors, the factor N seemed to be one of the susceptibility factors whose presence was needed by all analyzable tumors.

Thus we have found: that these eight mammary gland tumors from females of the highly inbred A race of mice are all carcinomata; that they are not dependent for growth upon the same number of susceptibility factors in all cases; that they are not dependent for growth upon identical factors; and that they are not all entirely dependent for growth upon the susceptibility factors carried by the individuals of the A stock.

GENERAL DISCUSSION

We have seen in this investigation that physiologically different types of carcinomata of the mammary gland may arise in females of a highly inbred stock. Furthermore, it is shown that these different types may occur even within the mammary gland of a single female of highly inbred stock.

The fact that these morphologically similar tumors from individuals of an inbred stock are physiologically different is of particular interest. This is especially so when spontaneous tumors from the same individual show physiological differences in their reactions during transplantation. In the case of female 13738 we have three such spontaneous tumors which were physiologically different from one another, as shown by their reactions during transplantation. It is certain that the differences in these three tumors would not have been detected had no transplantation work been carried out with them. Furthermore, it is probable that only a few of those physiological differences which could be demonstrated by transplantation experiments are actually detected. As evidence bearing upon this point we find that tumor 13738b has reacted differently from tumors 13738a and 13738c in individuals of both the D and the MD stocks and the individuals of the segregating hybrid generations of the $D \times A$ cross. Also that individuals of the N stock reacted differently toward transplants of tumor 13738b than did individuals of the D and the MD stocks.

These differences between the tumors from individuals of inbred stocks have been shown repeatedly to be genetic in character and to obey the principles of multiple mendelian factors. The dissimilarities which exist between morphologically similar tumors, from females of a highly inbred race of mice, and which are demon-

strated by the reactions between the hosts and the transplanted tumor tissues, are physiological differences.

These physiologically different tumors may have arisen from cells which were of different physiological constitutions. It is as though the cells of the mammary gland of an individual, such as female 13738, consisted of many physiological genotypes which were morphologically indistinguishable. It may be that the somatic cells of the body are divisible into a great many physiological types which are not detectable morphologically. If this were true, then even microscopic analysis of a tissue would give but a crude indication of the different types of cells that were present in the tissue.

Since cancer starts from a single cell or small group of cells, the presence of different physiological cell genotypes in the mammary gland of an individual might account for several physiologically different but morphologically similar mammary gland tumors arising spontaneously from the mammary gland cells of that individual.

If the above discussion is true, then morphologically similar tumors coming from physiologically different cells might easily react differently to the same therapeutic treatment. Some morphologically similar tumors which respond differently to radium do occur and are well known to radiologists.

As shown in the body of this paper, each of the tumors used gives good evidence of the functioning of multiple mendelian susceptibility factors. It is a significant fact that, although three tumors have arisen from the mammary gland of a single individual, and are all physiologically different from one another, all show an orderly genetic control of their reactions toward their hosts when they are transplanted into animals of genetically controlled stocks and their hybrid generations. This also holds true for all the tumors which occurred in the females of the highly inbred A stock, and which have been used for transplantation experiments.

We can see from the above that, even within an inbred stock whose individuals are theoretically very close to homozygosity for all factors involved, we get physiological differences within morphologically similar body tissues. Yet these differences are under the influence of orderly genetic control. This demonstrates beyond question the value of genetic analysis as a method of cancer research.

CONCLUSIONS

1. The carcinomata employed in this investigation occurred spontaneously in the mammary glands of females from the highly inbred A stock from Strong's laboratory, and their growth after transplantation is primarily dependent upon susceptibility factors carried by the individuals of the A stock and transmitted by them into their hybrids.

2. These tumors appear to need but few susceptibility factors for their continued growth after transplantation when compared with the experience of investigators who have used tumors of hybrid or of unknown genetic type.

3. The histologic structure of these carcinomata does not indicate their degree of malignancy.

4. The multiple factor hypothesis as the explanation of susceptibility to implanted tumors has been verified, and the use of genetically controlled stocks has been demonstrated to be of major importance in detecting and analyzing the physiological characteristics of transplanted tumors.

5. The use of unrelated inbred stocks and their hybrids is of value in detecting some of the physiological characteristics of a single tumor and in detecting and comparing some of the similarities and differences of these physiological characteristics of two or more tumors transplanted into the same hosts.

6. All of the tumors whose factor complexes could be analyzed indicate that the susceptibility factors carried by the individuals of the A stock are of two kinds: a general susceptibility factor N needed by all, and other susceptibility factors which are specific and characteristic for each tumor.

7. About 31 per cent of the non-susceptible D stock differ physiologically and probably genetically from the rest of the D race, for they allow transplants of tumors 13738b and 15091a to grow progressively.

8. It appears as though the growth of transplants of tumors 13738b and 15091a depends upon the presence of two dominant susceptibility factors carried by the A stock individuals *or* upon the homozygous recessive condition of a single factor found in some of the D stock individuals.

9. The distorted ratios obtained by transplanting these same two tumors into the individuals of the segregating hybrid generations from a (D $\times$ A) cross may be due to the presence of both of these types of susceptibility factors in the hybrid individuals.

10. A physiological difference exists between the investigated tumors which occurred spontaneously in females of the inbred A race, and even between the tumors of the same individual, as in the case of female 13738.

11. Two or more tumors which arise from the same tissue of an individual may need the same number of susceptibility factors, but these factors need not be identical.

12. Some fundamental change (probably in the nature of a somatic mutation) occurred in the tumor 17495a during the process of transplantation.

13. All the detectable physiological characteristics of these tumors appear to be under an orderly genetic control which is specific for each tumor.

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